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VOLUME 21 NUMBER 2

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An International Journal

Official Organ of
The American Phytopathological Society

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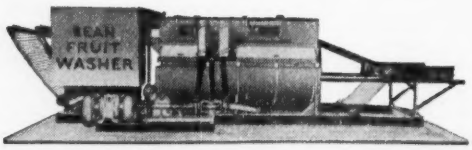

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PHYTOPATHOLOGY

VOLUME 21 NUMBER 2

FEBRUARY, 1931

STUDIES ON A RUST OF CLINGSTONE PEACHES IN CALIFORNIA

M. C. Goldsworthy and Ralph E. Smith[1]

The commercial canning of peaches and the production of fruit for this purpose constitute an industry almost entirely confined to California. Statistics presented by Wellman (38) show that since 1921 California has produced more than 97 per cent of the canned-peach output of the United States. During the past decade there has been a tremendous increase in the planting and total pack of clingstone varieties, accompanied by a considerable decrease in the output of canned freestone peaches. The following figures are taken from the California *Fruit News*, June 15, 1929.

Number of cases of peaches canned in California

	1921	1922	1923	1924	1925	1926	1927	1928
Freestone	1,633,418	1,314,597	872,676	963,621	1,198,314	817,319	320,812	163,830
Clingstone	4,162,849	7,844,912	6,591,335	5,366,598	9,258,587	13,654,758	10,829,681	14,811,606

Peach trees are susceptible to many diseases, among which that known as rust has become of great potential importance to the clingstone-peach orchards of California. During the three years previous to 1928 serious outbreaks of this disease occurred in various parts of the State, especially in that portion lying along the Yuba, Feather, and Bear rivers in Yuba and Sutter counties, which, because of the character of the soil and climate, is particularly adapted to the production of quality fruit and maximum tonnage. The region is devoted to the culture of the clingstone varieties and the crop is handled entirely through the canneries.

The disease under discussion has been known in this region and in other parts of California for many years (Scribner (33), 1887; Pierce (32), 1894) but usually on such a minor scale that its occurrence was thought to be of only passing significance as an indication of climatic conditions un-

[1] Acknowledgment is due to Professor W. P. Duruz (20, 21), whose spraying experiments on the control of peach rust were closely associated with this work.

usually favorable to the fungus but not likely to occur regularly or frequently. During 1925, 1926, and 1927, however, such serious losses resulted from this cause that the growers and canners became deeply concerned at what really seemed to be a new and serious menace to the industry.

This fungus disease, a true rust, has occasioned unusual alarm because it not only caused leaf injury and more or less defoliation, as is usual with rusts, but, particularly in 1926, entailed immense losses of fruit, the peaches being marked with rust lesions and rendered unfit for canning. Many growers thus lost their entire crop of certain varieties. The fact that the new, so-called midsummer clingstone varieties, extensively planted in recent years, have been more susceptible to this disease than the older varieties increased the seriousness of the situation.

Since 1927 almost no loss has been caused by rust in peaches. It therefore is natural to assume that the outbreak described was of a sporadic nature and that the reasonable expectation regarding this disease is one of only occasional and irregular occurrence. Experience with other plant diseases, however, has shown that it is not safe to rely too strongly on this assumption without the reassurance of a thorough knowledge of the nature of the disease and methods for its control. The fundamental relation of rainfall and relative humidity at certain seasons to the development of rust, thus explaining its sporadic occurrence, is brought out in this paper.

DESCRIPTION OF THE DISEASE AND THE CAUSATIVE FUNGUS

Cursory examination shows that this disease is caused by one of the true rust fungi (Uredinales), characterized most commonly by the production of an abundance of urediniospores.

Leaf phase (Fig. 1). This is the most frequent form of the disease and may become at times so severe that complete defoliation occurs. The leaves never become entirely covered with rust spots, but, as a rule, these are either scattered irregularly over the entire surface or confined to portions of the leaf. The areas first attacked become evident as pale, yellowish-green spots clearly defined on both surfaces. These spots are angular in outline and apparently are limited by the larger veins. Later these spots take on a bright yellow color, and the lower surfaces, rarely the upper, become conspicuous by the presence of dusty, dark brown rust sori in the central portion of the chlorotic area. These sori are filled with the cinnamon brown urediniospores.

When lightly affected, the leaves do not prematurely drop but remain on the trees until the normal fall in early winter. When heavily affected, that is, when most of the leaf surface is occupied by diseased areas, abscis-

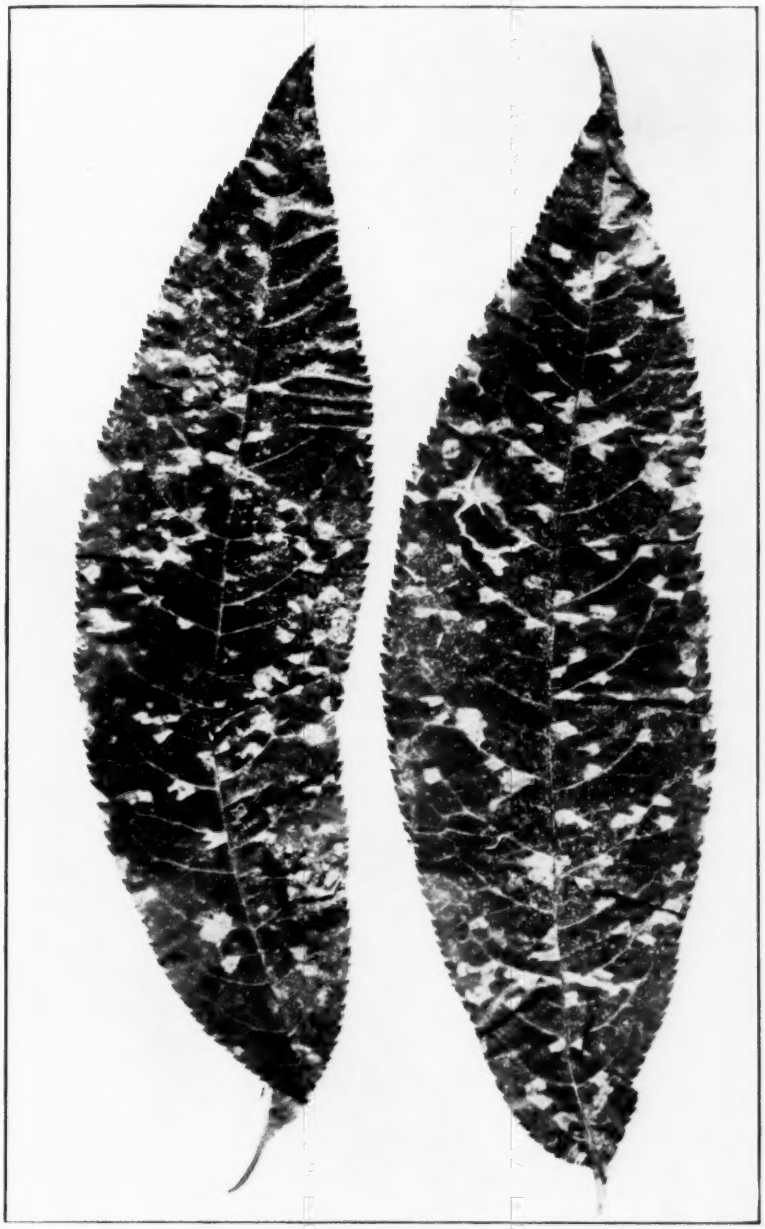

FIG. 1. Peach rust; leaf phase.

sion may occur and a partial to almost complete defoliation result. The effect of the organism upon the host is local and confined to a relatively small area of the mesophyll. The affected area does not collapse for some time and the first indication of a decline is the disappearance of chloroplastids which, no doubt, accounts for the color change in the rust spots on the leaves. After the fungus fruits the affected regions appear, for a time, greater in thickness than the adjacent normal portions of the leaf. This phenomenon is due to the presence of intercellular mycelium and a stromatic layer of pseudoparenchymatous tissue directly beneath the epidermis and not to an increase in the number of cells. Later these central portions

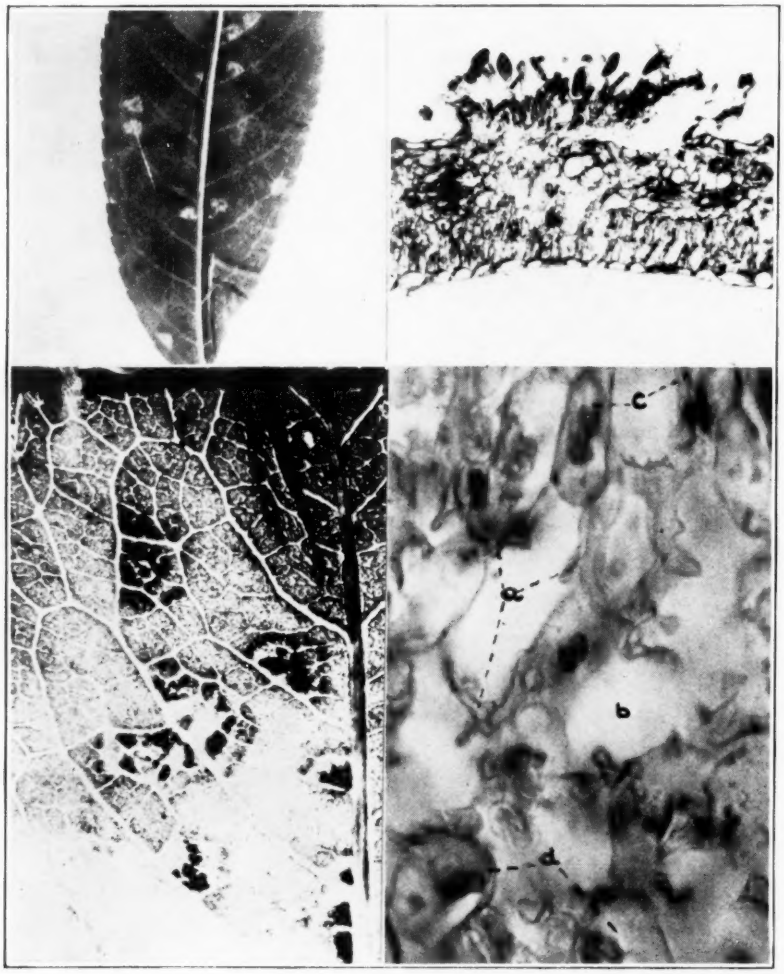

FIG. 2. Peach rust on leaves and sections of leaves showing sorus and affected mesophyll.

of the affected regions shrink, the cells take on a plasmolyzed appearance and during periods of high temperature they dry out, forming in some cases dead areas. The fungus continues, however, to live in the marginal tissues of the affected areas and later, as the cool weather of the fall comes on, new rust sori are formed in this part of the old lesion. The dead areas do not abscise, as in shothole diseases, but remain firmly attached.

Mycelium. The fungus invades the tissue with the formation of intercellular mycelium, the filaments containing many nuclei and with a relatively small diameter. In no case examined was the diameter of the hyphae greater than 8 μ and in most cases it was less and exceedingly irregular. The fungus forms abundant haustoria, which attain various sizes. Many of these are found in the cells and as many as six have been seen in a single large cell, such as is found in the peach hypodermis (Fig. 2, a).

Urediniospores. Spore production is characterized by the formation of a stromatic layer of pseudoparenchymatous tissue which, in the leaves and bark, is formed directly beneath the epidermis and, in the fruit, at various distances beneath the epidermal layer. From this layer mycelial strands are formed, woven into a more or less compact superstructure, at the apex of which each strand forms either a paraphysis or a urediniospore (Fig. 3, A).

The paraphyses are long, thin cells, bulging at their apices into rounded ends with a thickened brown covering. These vary in size, the longest examined measuring 32.6 μ, the shortest 17.6 μ. The number of paraphyses in a sorus appears to depend upon its maturity, for in young sori more of these structures are present than in the older, and, as a matter of fact, in the later cases it is quite common to find no paraphyses.

The urediniospore is borne on a stalk consisting of a basal cell and a short pedicellate cell. The urediniospores are cinnamon brown in color, mostly truncate, often elliptical and rarely isodiametric, made conspicuous by a thick, smooth apical tip of varying dimensions, sometimes covering as much as a third of the longitudinal diameter. They are heavily echinulate except at the apex, usually sharply constricted at the base, pedicel not adhering, heavily filled with granular protoplasm and oil inclusions and with pigment apparently contained in the outer covering. There are two germ pores, usually located beneath the apical cap and opposite each other. Measurements of 1,000 spores from various sources gave an average maximum-minimum of 33.6–12.2 x 14.4–9.0 μ. Typical urediniospores are shown in figure 4, B.

Bark phase. The bark phase of the disease (Fig. 4, A) is a relatively uncommon one in the Sacramento Valley and because of this has evaded regular observation. On many small, current-season twigs, marked as

FIG. 3. Sections of diseased tissues. A. Portion of leaf sorus, X800. a, paraphyses; b, urediniospores. B. Section of hypodermis of fruit. a, haustoria of rust fungus.

being noninfected in the fall, spore pustules have been found early in the spring before the leaves unfold and at about the time when the buds are swelling. The fruiting bodies break through the bark at this stage without forming an apparent lesion. The epidermis of the host becomes raised slightly, the tissue directly adjacent being slightly water-soaked in appearance, and finally the urediniospores and paraphyses break through the bark. Later, as the twig grows in circumference, these areas split open lengthwise along the twig, allowing free egress of the spore masses. The lesions formed in the bark are superficial and localized and appear not to affect the host to any particular degree and in growing twigs soon are covered by new bark. In fruiting twigs, however, where the centripetal growth is not great, the lesions remain a menace in that a small percentage harbor a perennial mycelium. In these cases sporulation occurs again the following

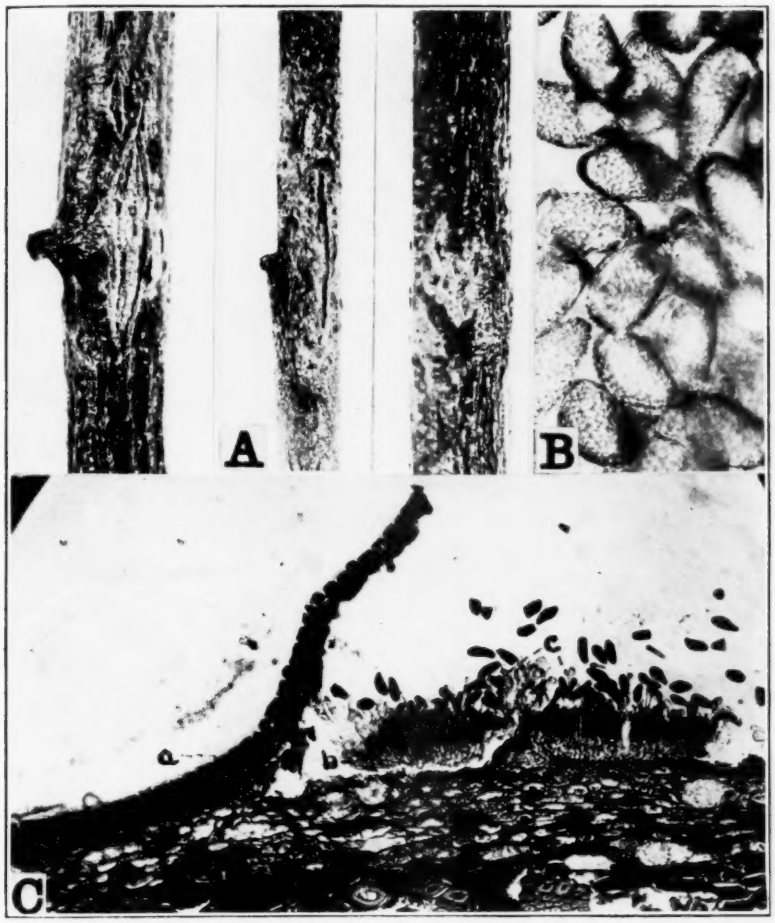

FIG. 4. A. Rust sori on bark of peach. B. Urediniospores. C. Section of bark sorus.

(second) spring along the margins of the old lesions. Microscopic examination of the affected regions reveals the fungus confined to the parenchyma of the cortex, the mycelium is intercellular, and haustoria are found within the parenchymatous cells. The fungus fruits by forming a stromatic layer of pseudoparenchyma just beneath the epidermis or bark of the host. From this there forms a mass of loose-woven hyphae upon which paraphyses and urediniospores are differentiated. The slight blistering or swelling of the surface of the host, observed at the point of pustulation, is not connected with an increase in the number of cortex cells but is due to the accumulation of the stromatic layer of the fungus below the epidermis. The paraphyses and urediniospores are similar to those of the leaf sori.

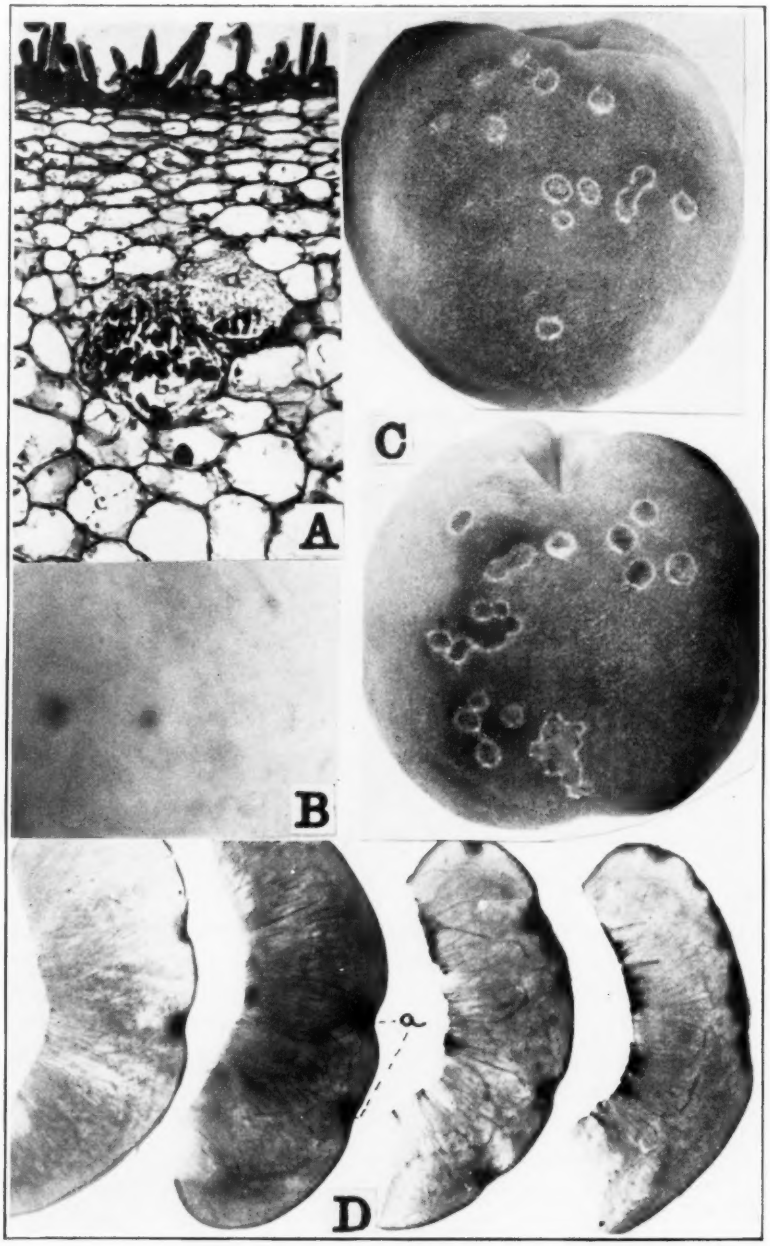

FIG. 5. Peach rust on fruit. A. Internal sorus. B. Mature fruit lesions. C. Young fruit infections. D. Cross sections of fruit lesions.

Fruit phase (Fig. 5). Fruit infection occurs only when germinating conditions are at an optimum for the fungus at a relatively late stage of fruit maturity. The areas affected appear first as water-soaked, dark green spots (Fig. 5, C). Growth is arrested, the spots become sunken as the adjacent tissue continues to put on new increment, and the central portions remain somewhat greener than the margins, becoming deep yellow when the lesions are older. On approaching maturity the normal pigmentation of the fruit occurs and the rust spots stand out vividly against this background. Urediniosori are formed in many, but not all, of the diseased areas on the fruit and these occur as dark brown, dusty areas in the very center of the region. In many cases, however, the sori never reach the surface, for they are formed within the hypodermis, sometimes covered with as many as 24 cell layers of this tissue, which, as the disease progresses, becomes tough and leathery but not killed. Figure 5, A, shows a typical case of submerged sorus in the fruit. The formation of these stromatic masses within the hypodermis and not, as in the leaf and bark, below the epidermal layer is no doubt a result of new increments being laid down by an active cambial region above the area originally affected.

The fungus mycelium in the fruit of the peach is intercellular and many haustoria are formed within the cells. At first the affected cells show no characteristic changes, but plasmolysis is noted later, the cells become collapsed, and a tough leathery region is formed which clings tenaciously to the adjacent normal tissue, causing, when the fruits are lye-dipped for peeling, a shallow-seated blemish which renders the fruit undesirable for canning (Fig. 5, D).

Accial stage. Not observed.

Telial stage. Not found, either on peach or any related host in the Sacramento Valley.

EPIPHYTOLOGY

This rust, so far as determined, is confined in the Sacramento Valley region to varieties of clingstone and freestone peaches and has not been found on apricot, prune, plum, almond, cherry, or on any alternate host. The overwintering bark stage will be taken as the starting-point in the discussion of epiphytology. The observations cover a period of two entire years, with an additional one in which the rust had completed its development. This comprises the latter part of 1926 and all of the seasons of 1927 and 1928.

In the summer of 1926, after a survey of the various infected orchards as to orchard management, irrigation practices, presence of wind barriers, relation of infected orchards to the Feather River, and varietal susceptibility had been made, the amount and time of rainfall and the occurrence of

previous infection were indicated as the principal factors in the development of the disease. Methods of irrigation, as, for instance, flooding compared with furrowing (which appeared at first as being of some consequence), appeared not of any great concern in relation to continued infections. It was noticed that open ditches in which water continuously flowed slighly favored local infection but that this was of little practical importance.

The most important observation in this preliminary investigation was that of the bark phase of the rust which at this time, August, 1926, was found on the previous year's twigs but not on the current season's wood. As a result of this, a careful investigation was made as to the inception of this phase for it was early thought that this might furnish the means of overwintering of the fungus. From the manner in which successive periods of leaf infection occurred it appeared evident that such infections were correlated with rainy periods during late spring and early summer. At the same time, however, although many twigs were examined, no new bark infections could be found.

The winter of 1926–27 arrived with still no findings as to the formation of the new bark phase. Soon after the opening of the buds in the spring (1927) many leaf infections occurred and it was soon found that nearly all of these primary infections were adjacent to bark lesions which had recently appeared on the twigs of the previous season. In a few cases, where single lesions were found on leaves with no new bark lesions nearby, the infection appeared to be due to spores disseminated from old bark lesions, of which many were present on two-year-old wood.

In the fall of 1927 many twigs were labeled and examined throughout the winter and during the spring of 1928, with the result that two types of bark lesions were discovered—one that forms early in the winter and fruits early in the spring and another that sporulates later as the buds are swelling. The origin of this last type, which is by far the more common of the two, is not positively known but, judging from the twig observations just mentioned and indirectly from the results of spraying experiments, it apparently results from twig infections in the early fall, in which the fungus overwinters in the mycelial form. In the spring the fungus breaks out through the bark with groups of uredinia and the spores cause infection in the near-by leaves as they unfold.

This primary leaf infection, originating in bark lesions, is characterized by the infection of all the adjacent leaves and these are found below and above and to the sides of the bark lesion. Rarely does the infection spread more than two feet from the bark pustule. The leaves are usually heavily infected. Spore production in these primary foliage lesions occurs in from three to four weeks after the leaves come out, and, if conditions are favor-

able, secondary infections occur on other leaves as soon as the spores become mature. This crop of spores gives rise to further leaf infections and these are of importance to the grower, in that they are the source of the fruit infections and resultant loss of the crop.

As fall approaches, many of the leaf lesions that have remained dormant all summer become active again and sori are produced along their margins. As these sori mature and the fall rains come on, infections apparently take place in the bark of the current season's wood, which has thus far remained free of rust, and also occasionally in that of the two-year wood. These lie dormant throughout the winter and give rise to urediniospores in the spring, which are responsible for the leaf infection, and the life cycle begins anew.

Another and important phase of the bark infection was discovered during the spring of 1928. At this time, on trees that had received careful and timely sprays and showed no sori on the last season's twigs, leaf infections were encountered which had all the typical symptoms of being associated with bark pustules. Examination of two-year-old fruiting twigs revealed the presence of old bark pustules which had remained dormant during the summer and fall, and had become active at this time, giving rise to a new crop of urediniospores at the margin of the old lesions. This finding was of great importance in that it accounted for the observed tendency of the organism to escape treatment. This made the problem of eradication doubly hard, since it required not a single season but at least two for that purpose.

In summary, the fungus overwinters as urediniospores in the bark sori of the previous year, or as recent infections on bark, or by the renewed activity of old bark infections; the adjacent leaf surfaces are infected during the period following initial growth in the spring; subsequent infections occur on other leaves or on fruit but not on bark; the fungus ceases activity during the dry months of the summer; activity of the leaf lesions follows during the fall months and finally new infections occur on the bark of current season's wood during the fall, which remain dormant and invisible until spring. Several previous investigators have observed the bark phase of the disease on peach but they state, or leave the inference, that the infection takes place in summer along with that of the leaves, which was not the case in the Sacramento Valley during the period of this work.

EPIPHYTOLOGICAL FACTORS

Moisture relations, maturity and age of spores, temperature, and time are the factors of especial interest in the case of the peach-rust fungus, all playing important parts in the activities of the organism and its infection of the host. The effects of the above factors upon the germinability of the spores and upon the incidence of disease are included in the following

remarks. The order of their importance, judging from observations and experiments, is the same as that given above.

Relation to moisture. It became evident, after a detailed study of the disease as to its occurrence upon leaves, fruit, and bark and examination of precipitation records, that moisture played an important part in the life history of the organism in this district. Infrequent rains or rainy periods characterize the spring months in northern California, and in most years not a great deal of rain falls after the first week of April. The history of leaf infection in any one spring and early summer can easily be traced by the distribution of rusted leaves along the twigs. The usual case is to find most of the leaves affected close to the base of the twig. If the spring rains extend over a relatively long and late period, as they were in the spring of 1926 (Table 1), the leaves for some distance along the twigs become infected. It happened in this particular season that sufficient rain fell between May 4 and 9 to produce a period of late infection; the correlation could plainly be seen in the leaves.

There was no indication during this particular spring and summer, except in special locations, of another advance or period of infection. The epidemic during this season was severe and many orchards were almost defoliated and much fruit was damaged. Two very distinct rust attacks were noted in all orchards examined except one and that was located in the river bottom between large levees, where the top soil was moist each morning and heavy dews prevailed throughout April and May, causing a continuous but not severe infection on the leaves for a longer period than in the other orchards. It was apparent, however, even in this location, that fruit infection had not resulted from this continued leaf infection but was correlated with the heavy, continuous rainy period in May.

In 1927, the initial rust attack was light and very few orchards showed heavy early infestation. However, in the few orchards with which we experimented, the check trees showed a fair amount of rust. The season was two to three weeks later than the previous one and no sustained precipitation occurred. The primary rust had developed on the leaves surrounding bark pustules, but no secondary leaf infection was noted until the first week in June, when a large amount of leaf rust appeared. A rainy and cloudy period, in which the precipitation amounted to 0.12 inch, occurred during April 25 and 26, and this probably accounted for the secondary infection noted above. On May 27 and on June 7 considerable rain fell (0.82 and 0.67 inch). By the first of July many reports of infected fruit, widespread as to their sources, were received. There was an abundant secondary leaf infection in the affected orchards, evidenced by small pin-point rust spots on newer leaves together with the spotting of a great deal of fruit. It so happened that the rain of May 27 was irregularly distributed and

TABLE 1.—*Daily rainfall data of the months of September to June, inclusive, for the years 1925 to 1928, inclusive, at Marysville, California*[a]

1925–1926

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	Total
Sept.						.11																									0.11
Oct.											.02																				0.02
Nov.		.09	.06						.54	.15	.17	.20			.10														.21	.26	1.78
Dec.	.91																.69	.17													1.77
March																															.0
April			.30	2.89	.55	.20	.58	.25									.14														4.91
May				.20	.39	.18	.20																								0.97
June																															.0

1926–1927

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	Total
Sept.																				Tr.											Tr.
Oct.	1.20								1.28	.11																					2.59
Nov.										.29	.30	.24						.33	.75	1.70	.70		1.21	.26	1.00	1.12		.44			8.34
Dec.	Tr.	.24															.31			.15		.05			Tr.						0.75
March								.53	.09			.70	.79															.10		.62	2.83
April	.60		.14					.12																		.12					0.98
May																			.08	.04							.82				0.94
June							.66																								0.66

1927–1928

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	Total
Sept.																		Tr.													Tr.
Oct.																										.62				1.04	1.66
Nov.						.10	.60	.75	.75				.37							.43					.39						3.39
Dec.									.60			.05	.25											.95	.05			.17			2.07
March		.11		.25			.06	.32														.97	1.81	.56		.65					4.73
April	.20	.68									Tr.					Tr.	Tr.														0.88
May																															.0
June																															Tr.

[a] From climatological data of U. S. D. A. Weather Bureau.

one orchard with which we experimented was exposed only to a trace of rain. The June 7 rain, however, occurred with full force. This orchard had been sprayed immediately before this heavy rain (June 3–6). All the trees manifested severe leaf infection at the time the spray was applied. Nonsprayed check trees gave almost 100 per cent infected fruit, while the sprayed trees showed from 8 to 10 per cent, demonstrating the relation between fruit infection and the rain of June 7. In this case the evidence is completely in favor of the conclusion that rains are necessary for fruit infection. What had been supposed to be a mild rust season, through this single period of rainy weather, turned out to be an important one and brought the matter of moisture relation forcibly to the attention of the fruit growers.[2]

In the spring of 1928, when a heavy precipitation was noted in March (4.73 inches) and during the first two days of April (0.88 inch) considerable primary leaf infection occurred. No rain fell after April 2, and no secondary infections occurred on leaf or fruit. Not a single spotted fruit was observed during the spring or summer.

During these three years another period of infection occurred in the fall, when a great many leaves became rusted. During this time considerable dew or precipitated moisture accumulated at night in the orchards and, because of the shorter days and lower temperature, the time period for germination was increased. In other words, the fungus found optimum moisture conditions and, as a result, considerable leaf infection occurred.

The three seasons of observation strongly indicate that periods of considerable rainfall or dew accumulation are necessary for secondary infection of leaves and fruits. As to the relation of moisture to bark infections, it has already been noted that in the three seasons of our observations no bark infection occurred during the summer periods of leaf infection. It seems probable that rainy periods or prolonged periods of heavy dew are necessary, provided inoculum is present, for bark infection. It appears from the success of spraying experiments, in which early fall applications controlled this phase, that the bark infections occur in the fall and these are correlated with the above moisture periods.

Urediniospore germination (Fig. 6). As infection of the host is an aftermath of spore germination, considerable laboratory work was done in testing the relation of various conditions to the germinability of the spores of the parasite. A bulging of the spore wall is the first symptom of germination. A light-colored area in the cinnamon-brown spore membrane

[2] In 1930 a period of cool, humid, and rainy weather, ideal for the development of peach rust, occurred between April 23 and May 16. That the disease did not appear was apparently due to the lack of inoculum resulting from the absence of rust in 1928 and 1929.

becomes hyaline as the germ tube emerges. The wall of the germ tube appears always in continuity with that of the parent spore and at times the basal portion of the germ tube may be covered with the spines which so conspicuously mark that of the mother cell. The germ-tube wall is hyaline and, as a rule, constricted at the point of emergence. Sometimes this constriction may extend outward from the spore wall for some distance. (Fig. 6; b, j, and m). At other times it is confined to that portion near the germ pore. In a proper environment the germ tube grows rapidly, in one case at the rate of 9 μ in ten minutes, in another 13 μ in eight minutes. The tubes are hyaline and contain finely granulated protoplasm, more or less homogeneous throughout, with occasional spherical oil globules dispersed throughout the plasm. What appear to be nuclei are always located at the very tips of the growing hyphae. Branching occurs by budding and in no case have we found septa.

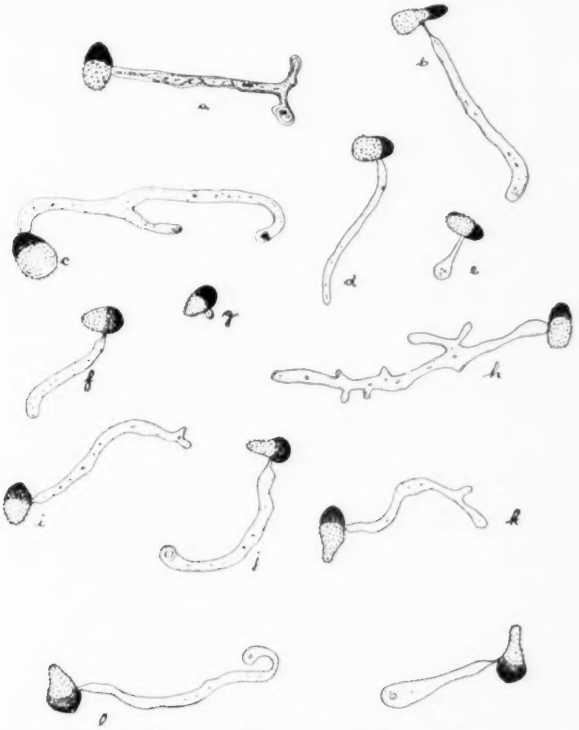

FIG. 6. Germinating urediniospores of the peach-rust fungus.

The phenomenon of branching apparently is associated with pressure and concentration, and this occurs after the apex of the germ tube has been arrested in its development. This allows a concentration of proto-

plasmic material with a resultant bulging at this point. If relief is not attained by budding, the swollen apex will burst and this appears to be the fate of many germ tubes in germination studies. If growth is continuous germ tubes reach considerable lengths, some over 700 μ having been observed.

Relation of humidity to germination of urediniospores. The question of the moisture condition necessary for infection in the field has been shown to be correlated with reasonably long periods of saturated atmospheric conditions, such a period being one in which a film of precipitated moisture is present on the under surface of the leaves. In the laboratory such a condition is easily reproduced and can be obtained either by placing the spores directly in contact with a film of water or by placing them in a saturated atmosphere, such as is produced in a closed chamber. It was found that the latter method furnished a maximum oxygen tension without which spores appeared not to germinate unless in a stage of complete maturity. It was noticeable in our germination experiments, especially those of 1926 in which relatively old material was used, that the germination of spores on water drops was correlated with their age and that the germinability of these same spores, when placed directly in a saturated atmosphere in which there was a maximum oxygen tension, was considerably greater. Keitt and Jones (26) reported the same phenomenon as occurring with the conidia of *Venturia inaequalis* (Cooke) Aderh. Allen (1) reported in the case of the urediniospores of *Puccinia glumarum* (Schm.) Eriks. and Henn. that rain water and not tap or distilled water favored germination. This may have been due to oxygen tension.

Considerable data relative to the effect of moisture on the germinability of urediniospores occurs in the literature. The differences between species in this relation appears to be slight and it is the general opinion that films of precipitated moisture are necessary for germination. The works of Melhus and Durrell (30), Peltier (31), and Duggar (19) are particularly suggestive in showing the importance of this factor in the life cycles of rusts. The present findings in the case of the urediniospores of the peach-rust fungus are that either a saturated atmosphere or a film of precipitated moisture is ideal for germination. When the spores are immersed in distilled water germination is arrested; but, when floated on water drops, germination proceeds within three hours if the spores are mature. Table 2 shows the relation of relative humidity to germination. It should be clearly understood, however, that it is quite difficult to draw a distinction between films of moisture, such as one uses in hanging drops, and those which must occur when a saturated atmosphere is used. In the latter case the film envelops the spore but in the former the spore appears to float on the surface, being in contact with the film at one point. It is true, how-

ever, according to Peltier (31) and Melhus and Durrell (30) that there is a difference, as they reported no germination in saturated atmosphere for grain rusts. It is not clear what differences are responsible for this, but possibly the nature of the spore wall and especially its wax content may influence this phenomenon. Melhus and Durrell show that paraffin wax was favorable to an increase in germination and we have demonstrated, as shown in table 13, that light oils stimulate germination even so far as to allow spores to germinate when the untreated ones did not.

TABLE 2.—*Effect of relative humidity upon germinability of urediniospores of peach-rust fungus. 22° C. for 12 hours*

Relative humidity	H_2SO_4 specific gravity	H_2SO_4 percentage	Spores counted	Spores germinated
1.5	1.754	82.0	2420	0.0
10.5	1.569	66.0	1879	0.0
18.5	1.503	60.0	2321	0.0
42.0	1.380	48.0	3287	0.0
58.3	1.300	39.0	2578	0.0
70.4	1.250	33.4	3168	0.0
85.7	1.170	23.5	2988	0.0
95.0	1.090	13.0	3560	0.0
97.5	1.050	7.37	4267	0.0
98.7	1.030	4.49	3897	0.0
99.1	1.020	3.03	4124	0.0
99.5	1.010	1.57	3800	0.0
100.0	1.000	0.00	4688	1988.0

Discussion of moisture relations. Extensive peach-rust infections throughout the three years of observation were clearly connected with rainy periods or with situations having heavy and continuous dews and not with periods of irrigation.

If irrigation practices were responsible for differences in infection this was not noticeable in the places examined, as just as much rust occurred in nonirrigated orchards as in furrowed and flooded situations. Relative to the last point it is interesting to note that there have been certain cases in which secondary infections have resulted on leaves attached to low-lying branches adjacent to open ditches or where, because of faulty drainage, accumulations of irrigation water occur. In these instances we are of the opinion that the cumulative effect of many saturation periods favored germination. Probably, during this time, a certain percentage of spores germinated and caused leaf infections. In any case this type of infection is not severe and the lesions occur on only a few leaves. That irrigation is

not responsible for fruit infection was clearly demonstrated during the 1928 season, as no fruit rust occurred in any of the situations referred to above; in fact, no secondary rust on leaves occurred, except in the above special cases. Irrigation during times of rainfall might cause some increase in the length of the saturation period and thus have a direct influence on infection.

Laboratory studies demonstrate that precipitated moisture is essential for germination of the urediniospores and supplement the observations in the field.

Relation of age to germination of urediniospores. The age and maturity of urediniospores constitute an important factor in their capacity to germinate. The viability of urediniospores, in general, is of relatively short duration and, because of this, considerable mystery surrounds the survival of many of the species having only this form in their life cycles. In this respect, Barclay (6) was able to germinate urediniospores of *Puccinia coronata* var. *himalensis* for 4 to 5 months; Melhus and Durrell (30) found that those of *P. coronata* persisted for at least 55 days but that the temperature at which the spores were held constituted an important factor. At room temperature they remained viable only 30 days. Maneval (29) reviewed the literature relative to this and table 3 gives a synopsis of various findings. That various differences are noted, even with the same species, is due in part to the method of collection, age of material, temperature at which held, and the condition of host plants.

TABLE 3.—*Longevity of urediniospores of various species of rusts, after Maneval*

Author	Fungus	Longevity
		Days
Ward	*Puccinia dispersa* Eriks.	61
Fromme	*Puccinia coronifera* Kleb.	84
Reed and Holmes	*Puccina coronata* Cda.	77 (91?)
Melhus and Durrell	" "	55
Peltier	*Puccinia graminis* Pers., form 3	92
Frazier	*Puccinia helianthi* Schw.	182
Spaulding	*Cronartium ribicola* F. de Wal.	155[a]
Taylor	" "	Overwintered
Maneval	*Uromyces striatus* Schr.	176[a]
"	*Puccinia sorghi* Schw.	158[a]
"	*Puccinia coronata* Cda.	157[a]
"	*Puccinia menthae* Pers. var. *americana* Burr.	173
"	*Uromyces caryophyllinus* (Schr.) Wint.	185
"	*Puccinia amorphae* Curt.	89

[a] Average.

The viability of the urediniospores of the fungus under study is markedly variable and is greatly influenced by the source and the environmental conditions affecting the host at the particular time of collection. Peltier (31) has shown that the humidity at which urediniospores are held influences the period of viability. It was our experience that viable spores were obtained for a longer period from leaves remaining on the host than from material which had been removed from the trees, but this may have been due to the production of successive generations in the sori on the tree. In the case of bark pustules, many have been tested, and from these even late in the fall after the leaves have fallen a small percentage of viable urediniospores can be recovered. In the case of the bark sori some were wide open, due to growth of the host, while others were entirely submerged and covered with bark tissue, but the percentage of spore germination remained the same in both cases.

While it is interesting to know that mature urediniospores of this fungus overwinter to a slight degree in the bark pustules, this is not actually of great importance in the life history, since very little infection occurs from this source. The vast amount of urediniospores that develop in the leaf sori during the primary, secondary, and tertiary infections of the spring and early summer months is the important source of supply, and it is the relation of these urediniospores to the life cycle and the effect of maturity on them that are of interest to us in this respect.

Relatively speaking, the urediniospores of the leaf sori are short-lived. The continued recurrence of new sori in seasons of unusual summer rains, however, bridges these short cycles and to all intents and purposes longevity is not important in these years. It is during a relatively dry summer season that this factor is important, not so much in the life cycle, but economically. Table 4 shows the absolute values for certain sets of experiments. Urediniospores collected at intervals from diseased leaves on the tree of a known early infection period contained viable spores over a period of 6 weeks. In a later infection occurring in June, this period was shortened to 4 weeks. Urediniospores which remained attached to detached leaves showed 56 per cent germination when first tested, 29.8 per cent after 22 days, and, after 33 days, no germination (Table 5).
At no time did they show a larger percentage than at the beginning. Urediniospores detached from living leaves 17 days after collection gave 39 per cent germination, about the same value as that of spores from detached leaves. These values are only relative, since other factors are important in determining the number of viable spores one may find. Melhus and Durrell (30) have shown that urediniospores may, if detached from their sori, show an increased percentage of germination due to the after ripening of spores. The results obtained in germination experiments

TABLE 4.—*Longevity of urediniospores of peach-rust fungus in sori on living leaves upon the tree*

Date collected	Source		Age of (weeks)			Germinability		Per cent germinated
	Bark	Leaf	Bark	Leaf	Sorus	Spores counted	Spores germinated	
4–19–27		"		6	1	602	none	...
4–20–27		"		6	1	1280	none	...
4–28–27		"		7	2	3138	39	1.2
5– 6–27		"		8	3	1281	63	5.0
5–17–27		"		10	5	2516	387	15.4
5–24–27		"		11	6	1293	486	37.7
5–28–27		"		11.5	6.5	4738	3118	67.2
6–24–27		"		15.4	10.4	3673	38	1.0
7–28–27		"		20.2	15.2	1013	150	1.4
4–19–27	"		?			3280	1388	42.3
4–28–27	"		?			1586	879	55.4
5– 2–27		"		7.5	1.5	1081	2	Tr.
5– 9–27		"		8.0	2.0	3266	38	1.1
5–17–27		"		10.0	4.0	1281	16	12.5
5–19–27		"		10.0	4.0	2520	34	13.5
5–28–27		"		11.5	5.5	2096	553	26.8
6–22–27[a]				15.0	2.0	1672	10	.6
7– 6–27[a]				17.0	4.5	414	145	35.0
7–14–27[a]				18.0	5.0	616	282	45.0
7–28–27[a]				20.0	7.0	931	407	43.7
8–13–27[a]				22.5	9.5	680	14	2.0
6–22–28[b]		"		?	5.0	562	170	30.3
6–22–28[b]		"		?	5.0	508	193	38.0
6–20–28[b]		"		?	1.0	482	2	0.4
6–27–28[b]		"		?	2.0	931	106	10.2
7– 9–28[b]		"		?	4.3	586	393	67.7
7–14–28[b]		"		?	5.0	632	287	45.4
7–28–28[b]		"		?	6.0	581	30	5.1
7–11–28[b]		"		11.5	9.5	1664	54	3.2
7–11–28	"		11.5		11.5	931	75	8.0
9–21–28	"		17.2		17.2	1336	27	2.0
10– 8–28	"		20.0		20.0	1491	25	1.7

[a] New infections.

[b] New sori from old infections.

depend a great deal upon the manner in which the urediniospores are obtained from the sori. In any sorus at any given age there will be a difference between mature and immature spores. In most of the literature no reference is made as to the method of gathering the spores. Melhus and Durrell state that it made no difference whether the spores were shaken

TABLE 5.—*Longevity of urediniospores of peach-rust fungus on detached leaves, at 20–22° C.*

Date collected	Date tested	Spores counted	Spores germinated	Per cent germinated
9–29–27	9–29–27	5381	3013	56.0
9–29–27	10– 4–27	3669	1815	49.5
9–29–27	10–14–27	1762	705	40.0
9–29–27	10–21–27	3021	900	29.8
9–29–27	11– 2–27	927	0	0.0
10– 6–27	10–16–27	4638	1809	39.0

or whether they were scraped from the sori. In our experiments the spores first shaken off, presumably the oldest, gave the highest percentage of germination; those shaken off later germinated less; and those finally scraped off germinated not at all. Doran's (17) method of giving the percentage of germination at optimum as 100 per cent and raising the other percentages accordingly is satisfactory for any given sorus but it is obvious that each may have a different percentage even at the optimum. Regardless of method, in the case of urediniospores, only relative values are realized, as each source of material is different from the next. General trends can be arrived at through the accumulation of mass data, but it is obvious that absolute values are impossible unless the sori have a known history.

Longevity of spores becomes important in the life history of the rust fungus in that it operates to limit secondary and tertiary infection. Secondary infections are important not only in continuing the life history but also in determining the economic losses due to fruit infections. If conditions which favor secondary infection are absent, the further development of the fungus is checked by the death of the comparatively short-lived spores produced in the primary infection. Tertiary infections operate to carry the fungus into the fall of the year. It is obvious that in case an exceptionally abnormal fall season occurred in which rain was absent, this factor would be of the greatest importance, in that the fall infections of the wood would be reduced and overwintering would be more or less restricted to the older lesions.[3]

Relation of temperature to germination of urediniospores. The rust fungi in general have wide ranges of temperature at which the spores germinate. Doran (17) tested several species (Table 6) and found that

[3] This apparently happened in the season of 1929–30, when no rain fell until December 10.

TABLE 6.—*Spore germination temperatures of various rust fungi, after Doran*

Fungus	Temperature: Degrees C.		
	Minimum	Optimum	Maximum
Aeciospores of *Cronartium ribicola* F. de Wal.	5	12	19
Urediniospores of *Cronartium ribicola* F. de Wal.	8	14	25
Aeciospores of *Gymnosporangium clavipes* C. and P.	9	16	29
Urediniospores of *Puccinia antirrhini* Diet. and Holw.	5	10	20
Puccinia malvacearum Mont.	3	14	30
Urediniospores of *Uromyces caryophyllinus* (Schr.) Wint.	4	14	29

TABLE 7.—*Effect of temperature on the germinability of urediniospores of the peach-rust fungus. 12 hours or less*

Temperature, degrees C.	Spores counted	Spores germinated	Per cent germinated
1	3798	0	0.0
7	4296	0	0.0
8	2242	7	.31
10	1610	436	27.0
12	1561	436	28.0
14	3753	1554	41.1
15	2012	946	47.0
16	3637	1320	36.5
21	3762	2025	53.8
22	4320	1490	34.5
23	4750	2000	42.1
24	4710	2270	48.2
25	5388	2220	41.2
26	5640	1740	30.9
27	3816	584	15.3
27.5	4000	620	15.0
28	3340	672	19.7
29	4032	976	24.0
30	3915	684	17.4
31	3456	464	13.4
32	3496	744	21.0
33	3856	240	6.2
34	3600	64	1.9
36	4104	80	1.9
37	4104	64	1.5
38	3554	48	1.3
39	1872	0	0.0
40	4896	0	0.0

relatively low temperatures favored many rust urediniospores. Peltier (31) showed that high temperature lowered the percentage of germinating spores, while low temperatures favored it. Walker (37) pointed out, in the case of the smut fungus, *Urocystis cepulae* Frost, that optimum temperatures for chlamydospore germination also coincided with rapidity of length growth. Doran points out "that although urediniospores germinate over a wide range of temperature, there is a material degree of host infection only when the temperature is near the optimum."

The urediniospores of the peach-rust fungus have a wide range of germination temperatures (Table 7), the minimum being 8° C. and the maximum 38° C. with the optimum between 13° C. and 26° C. Melhus and Durrell (30) consider the length growth of the germ tubes as an indication of optimum. We have no comparative information relative to this for the peach-rust fungus, but, at 22°–24° C., the germ tubes appear to continue their growth at the maximum rate without injury. Below 14° C. and above 26° C. the germ tubes appear to be injured. At high temperatures the tips of the germ tubes burst soon after emergence, while others show branching. At low temperatures bursting does not occur but frequent branching is the rule, the germ tubes exhibiting all sorts of convolutions in contrast to the even, continuous growth at 22°–24° C. The temperature curve of germination (Fig. 7) appears to have a fairly rapid rise, after which it flattens out for some distance and drops off rapidly at first, then finally goes into a gentle slope. This is, in general, the trend of germination. Some discrepancies occur, but this is to be expected, since spore material is so variable. The field temperatures during the early season when rust attacks begin correspond, in general, to that part of the curve during which the period of optimum germination occurs. High temperatures are injurious to spores in that those kept at 40° C. and over for more than 12 hours, when removed to a suitable environment, never germinate. In the field, however, urediniospores seldom are subjected to such a high temperature for any length of time.

Where a fungus has such a wide latitude of favorable temperatures for germination, provided other conditions are at optimum, it seems logical that this factor would not be of great importance in natural infection. It is pointed out, however, by other workers that periods of infection coincide with the optimum germination temperature period. If this is so, a wide range of germination might, in the case of temperatures of either extreme being prevalent for any length of time, actually be effective in a control of the fungus, in that germination would result without infection, and thereby reduce the amount of inoculum.

Effect of fungicides on the germination of urediniospores. The literature on the subject of the control of peach rust, although scanty, indicates that Bordeaux mixture and lime-sulphur sprays may be used to advantage.

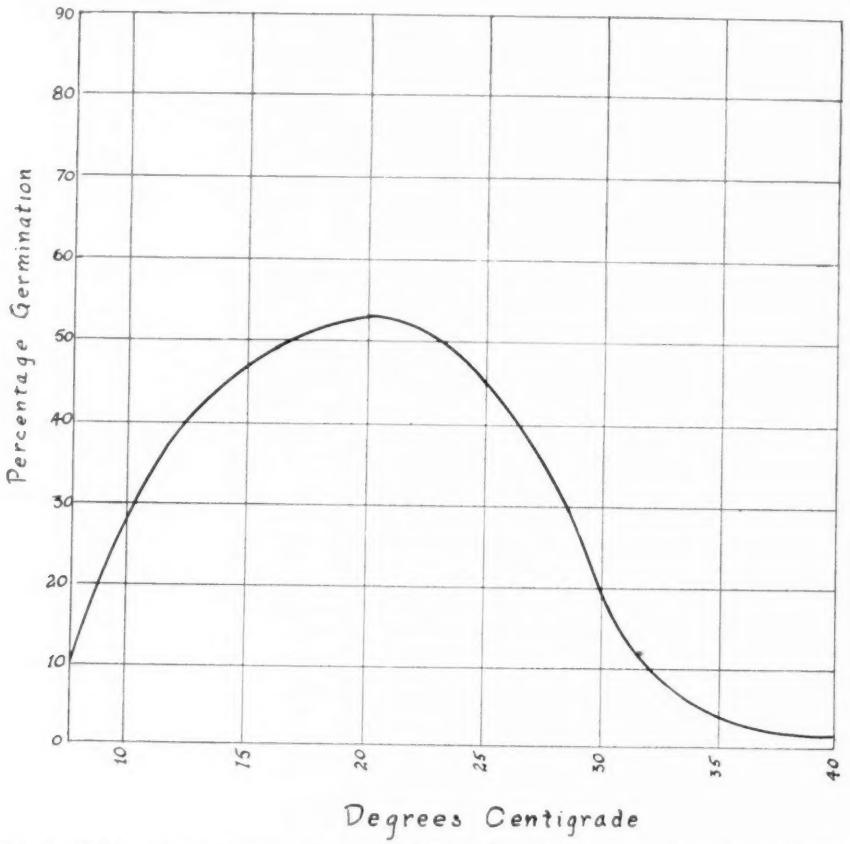

FIG. 7. Effect of temperature on germinability of urediniospores of peach-rust fungus. Twelve hours or less.

Galloway (23) reported the effectiveness of Bordeaux mixture in controlling plum leaf rust (*Puccinia pruni-spinosae* Pers.). He stated that Professor T. L. Brunke, at his suggestion, conducted a series of experiments in 1888 at the Texas Agricultural College with the view of finding a remedy for this disease. Brunke found that spraying with Bordeaux mixture in August gave good control. Those trees which were not treated lost nearly all their foliage, while those sprayed had lost only a very small percentage.

Pierce (32) reported, on the basis of one year's experiments, that Bordeaux mixture and other copper compounds applied at the pink stage of the bloom were of value in control of prune rust.

Fairchild (22) noted that, if the conclusions of Scribner and Pierce are well grounded, this is the first case to be established of a rust yielding to Bordeaux mixture.

Cunningham (15) advised the use of lime-sulphur sprays.

McCalman (28) reported that lime sulphur 1–50 or an application of Bordeaux mixture (2–2–40) kept down the disease.

Doran (17), testing urediniospores of various rust fungi, showed that these, as a rule, were very resistant to copper. Butler (12) and Doran (loc. cit.) found that dusting sulphur controlled Antirrhinum rust, and Smith (34, 35) showed that this material was effective against the asparagus rust in California. The Uredinales, in general, are, from all indications, comparatively resistant to copper and susceptible to sulphur compounds.

TABLE 8.—*Effect of copper sulphate on urediniospores of peach-rust fungus. 22° C. 12 hours*

Dilution percentage	Spores counted	Spores germinated	Per cent germinated
2.0	982	0	0
1.0	932	1	.1
0.5	868	0	0
0.25	943	0	0
0.125	841	41	5.0
0.06	531	40	7.5
0.03	1154	104	9.0
Water	2664	447	16.5

TABLE 9.—*Effect of various copper mixtures on urediniospores of peach-rust fungus at 22 C.*

Kind of dilution	Spores counted	Spores germinated	Per cent germinated
Homemade Bordeaux 4–4–20	1286	145	11.3
Homemade Bordeaux 2–2–50	1469	241	16.4
Homemade Bordeaux 5–5–50	6084	747	12.3
Homemade Bordeaux 8–8–50	1956	124	6.3
Commercial Bordeaux Ortho Brand 1–40	3463	1282	36.8
Commercial Bordeaux Ortho Brand 1–80	371	52	14.3
Borco Winter str.[a]	1710	408	22.9
Copper sulphate	1437	0	0.0
Water	6291	2206	36.0

[a] Ammoniacal copper compound.

Copper compounds. Table 8 shows the effect of copper sulphate on the urediniospores of the peach-rust fungus and table 9 the effect of various other copper compounds. It can readily be seen that the fungus is relatively resistant to copper. In field experiments, however, this was not so marked, and, although spores fully covered with Bordeaux mixture germinate when brought into the laboratory, there appears to be a distinct decrease in the amount of leaf infection in trees sprayed with this material as compared with unsprayed trees. The tests in the laboratory with spores treated with Bordeaux mixture show a considerable amount of germination, but this percentage is always below that of the checks. On microscopical examination one finds the spores along the edge of heavy Bordeaux mixture spots germinating first, and this is true also of those spores lying in the sparsely covered regions. The spores heavily covered with the colloidal material germinate last or not at all. On slides kept more than three days,

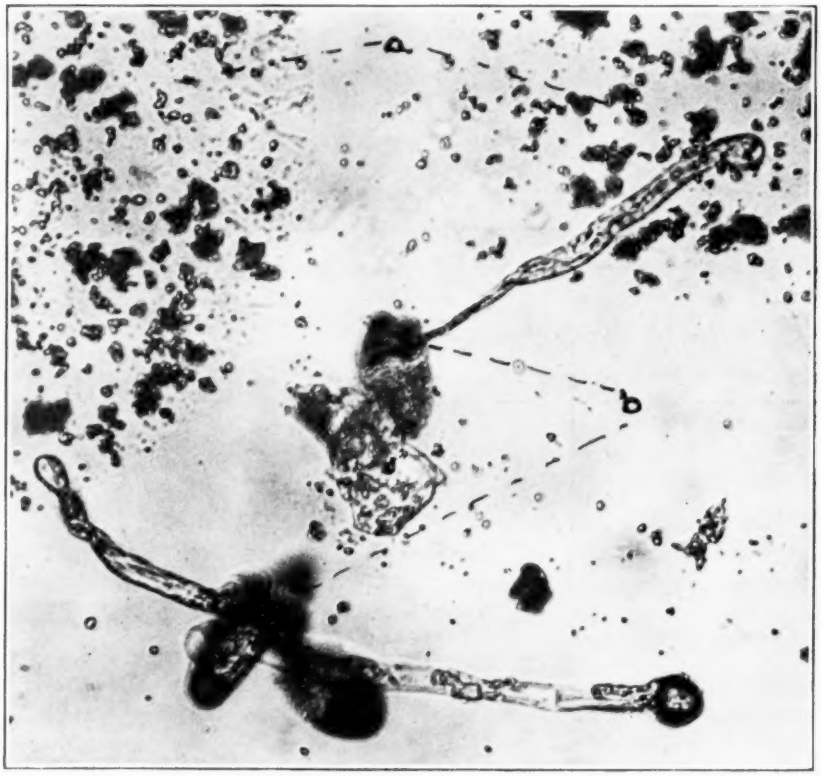

FIG. 8. Urediniospores of peach rust fungus germinating in a field of commercial Bordeaux mixture. Material sprayed upon spores on glass slide before germination.

the spores showed germination even in the heavily coated parts. Figure 8 shows germinating urediniospores in a field of commercial Bordeaux mixture of winter strength. This is not an unusual finding and is a common phenomenon with homemade and commercial Bordeaux mixtures and for ammoniacal copper solutions, as well.

Polysulphides. Polysulphides, as in commercial lime-sulphur solution, are very toxic to the urediniospores of this fungus, even in highly diluted solutions (Table 10). The effect on the spores and germ tubes, according to Goldsworthy (24), is direct, the sulphide reducing the protoplasm, which causes the death of the organism (Table 11). Figures 9 and 10 show the lime-sulphur-treated spores and germ tubes.

TABLE 10.—*Effect of lime-sulphur and powdered sulphur on urediniospores of peach-rust fungus at 22° C.*

Kind and dilution	Spores counted	Spores germinated	Per cent germinated
Commercial dry-lime-sulphur 14–100	4036	242	6.0
Liquid-lime-sulphur 1–100	1376	12	0.9
Liquid-lime-sulphur 1–50	3001	0	0.0
Liquid-lime-sulphur 1–10	3378	0	0.0
Powdered sulphur old lot	2060	198	9.60
Powdered sulphur new lot	8336	118	1.40
Sulphur-paste gas residue in water	1723	49	2.80
Water	12425	3516	28.30

Commercial dry-lime-sulphur (Sherwin-Williams) and sodium sulphide compounds are highly toxic to the urediniospores. In recent years liquid-lime-sulphur solutions have caused considerable burning to both foliage and wood of the peach, and the growers are becoming alarmed over the situation and are turning again to the use of copper compounds.

Powdered sulphur. Powdered sulphur has been extensively used in the control of fungi, especially powdery mildews and rusts. This material has been used against the peach-rust fungus by many growers, and the success attained has been difficult to determine, due to the varied conditions under which it was used. Sulphur apparently acts best at high temperatures and in still air and the lack of success of many growers may be attributed to

FIG. 9. Germinating urediniospores of peach-rust fungus sprayed with liquid-lime-sulphur 1–50. Sulphur particles on slide and in germ tubes.

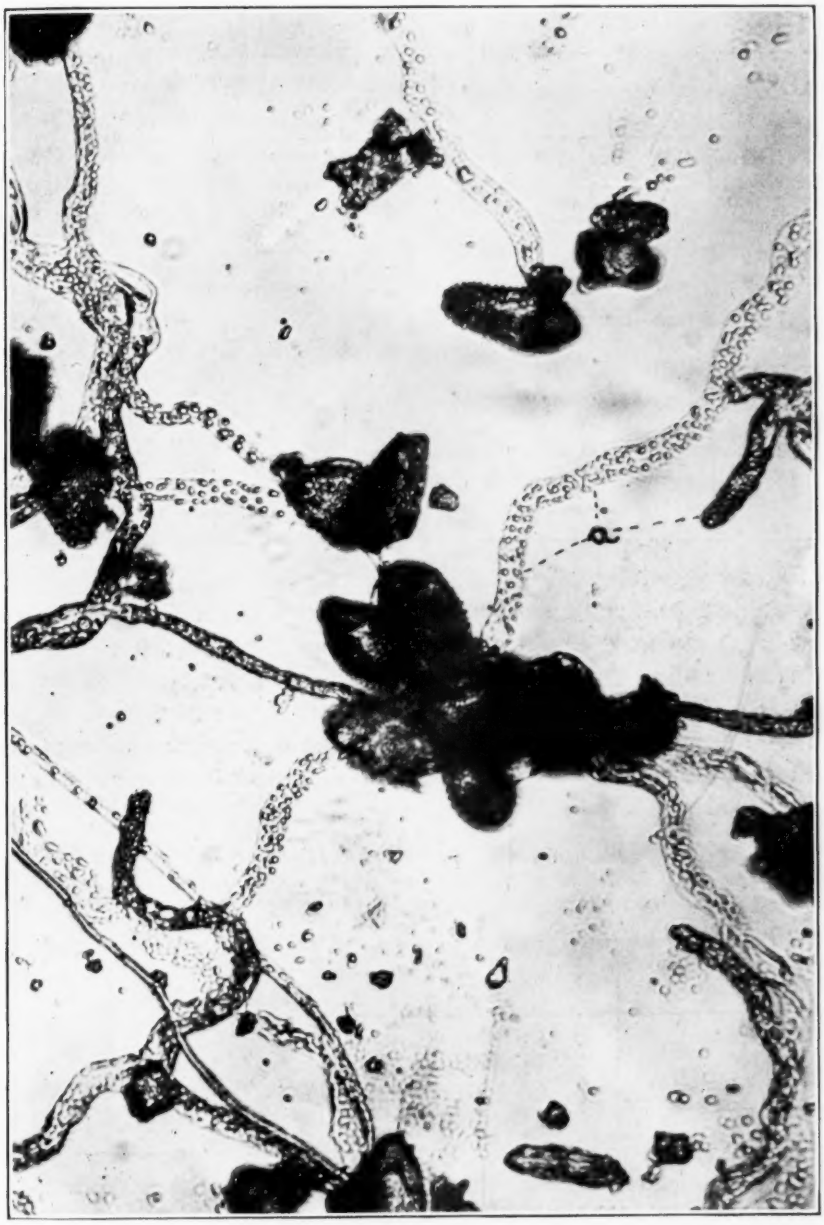

FIG. 10. Germinating urediniospores of peach-rust fungus sprayed with liquid-lime-sulphur 1–50. Sulphur particles removed from slide but retained in germ tubes.

TABLE 11.—*Effect of liquid-lime-sulphur 1–10 on the growth of germ tubes of the urediniospores of the peach-rust fungus at 22° C.*

Germ tube	Time sprayed	Length of germ tube (microns)			
		3 hours	6 hours	9 hours	12 hours
1	third hour	6	6	6	6
2	"	8	8	8	8
3	"	6	6	6	6
4	sixth hour	–	17.5	17.5	17.5
5	"	–	23.0	23.0	23.0
6	"	–	15.0	15.0	15.0
7	ninth hour	–	–	38.0	38.0
8	"	–	–	46.5	46.5
9	"	–	–	35.0	35.0
10	twelve hours not sprayed	–	–	–	61.5
11	"	–	–	–	82.0
12	"	–	–	–	77.0
13	twelve hours average of 12 tubes. Not sprayed	—	—	—	71.5

the fact that the temperature was not high enough at the time of application. With laboratory experiments, sulphur dust and a liquid mixture of a gas-house residue "sulphur paste" gave excellent results (Table 10). The sulphur dust and the paste were applied to spores on slides and allowed to germinate under the optimum moisture and temperature constants. In one case careful observations made during the 1928 season of peach trees dusted during June showed that an excellent control was effected (Table 12).

TABLE 12.—*Effect of powdered sulphur (orchard tests) on urediniospores of peach-rust fungus. Spores removed from leaf sori after trees were dusted and tested in moist chamber at 22° C.*

Date of applying sulphur to trees	Date of testing spores	Spores counted	Spores germinated	Per cent germinated
6–5–28	6–15–28	5658	14	0.2
6–5–28	6–17–28	4822	120	2.7
Check	6–20–28	631	50	8.0
6–5–28	6–21–28	5328	60	1.1

Other fungicides. The successful use of sodium silicofluoride, reported by Anderson (2) in the control of *Bacterium pruni*, the control of green storage mold of citrus fruits by the use of borax solutions, and the increasing use of mercury compounds as well as light oil sprays in the control of fungi prompted their use in laboratory tests. Table 13 shows the effect of

the various materials tested. In general, none of these, with the single exception of sodium silicofluoride, were promising and the light oil Volck stimulated germination. It is becoming the practice, since oils of various grades are coming into use for winter treatments, to combine various substances, such as Bordeaux mixture and sulphides, with them. For this reason we tried a mixture of sodium sulphide and a light oil emulsion in the proportion of 0.5 per cent sodium sulphide to 3 per cent oil emulsion in 1,000 cc. of water. A solution containing 2 per cent liquid-lime-sulphur and 4 per cent oil emulsion was also used. Almost complete inhibition of spore germination was effected in the laboratory but, in the field, these sprays were not so successful.

Sodium silicofluoride 0.25 per cent was used in field experiments but the injury to the foliage was so pronounced that its use was discontinued.

TABLE 13.—*Effect of various fungicides on urediniospores of peach-rust fungus at 22° C.*

Kind and dilution	Spores counted	Spores germinated	Per cent germinated
Sodium silico-fluoride 0.25%	5529	25	0.4
Borax solution 2.5%	496	22	4.5
Uspulun 0.25%	1951	120	6.2
Semesan 0.25%	1850	132	7.1
Volck oil emulsion[a] 3.0%	3560	1226	34.4
Lime-sulphur 2%, oil 4%	4433	57	1.3
Sodium polysulphide 5 gms.–30 cc. oil emulsion in 1000 cc. H_2O	1350	0	0.0
Water	12425	3516	28.30

[a] Apparently stimulated germination.

IDENTITY OF THE PEACH-RUST FUNGUS

The peach-rust fungus is commonly assumed to be identical with the well-known prune rust, *Tranzschelia punctata* (Pers.) Arth. (*Puccinia pruni-spinosae* Pers., *P. pruni* Pers.). This fungus has been reported in California for many years upon prune, plum, apricot, and almond, as well as upon the peach. (Scribner (33), Cooper (14), Pierce (32), Smith and Smith (36), Barrett (7, 8, 9), Blasdale (10).) A peculiar feature, particularly marked in the present instance of this peach-rust epiphytotic, is the varying specificity of the parasite as to host in different localities and the variation in the occurrence of the different spore forms on the various hosts and in different places. During the present work in the Sacramento Valley (1926, 1927, and 1928), considerable attention was paid to this phase of the subject. During this time the rust was never found in that

region on any host except peach. Even though prune trees stood immediately adjacent to badly rusted peaches no trace of the disease was found in the former host in any case examined. Regarding spore forms, none but urediniospores was ever found on peach trees in the Sacramento Valley, and many thousands of leaves and sori were examined.

In the Santa Clara Valley, south of San Francisco Bay, a very different situation exists relative to what is supposedly the same fungus. In this district, where prunes, plums, peaches, and apricots are extensively planted, rust develops late in the season almost every year upon the leaves of nearly all the prune and *domestica* plum trees. In this case the fruiting bodies, so far as determined, are all teliospores. Rust is never conspicuous upon peach or apricot and, during the years of the investigation herein reported considerable attention was paid to this point. Many cases were examined of peach and apricot orchards directly adjacent to prune orchards where the lower side of every leaf of the latter was covered with teliosori of the rust. Not a single pustule was found on peach or apricot. This reversal of conditions as to the comparative occurrence of rust on peach and prune in the Sacramento and Santa Clara valleys is most complete and striking, together with the predominant, if not exclusive, development of urediniospores on the peach in the Sacramento Valley and teliospores on prune and plum in the Santa Clara Valley. On apricot no rust was found in either of these districts during the period of this work. In 1927, however, a severe outbreak of apricot rust occurred at Arroyo Grande, a small valley opening directly on and within one or two miles of the ocean with a very cool and humid summer climate. This is in San Luis Obispo County at a point about midway between San Francisco and San Diego. Such an outbreak is not an uncommon occurrence in this locality. The rust develops on foliage and fruit during spring and summer with both urediniospores and teliospores.

Turning to the literature, Scribner (33) reports this rust fungus from California and other States on cherry, peach, apricot, and plum, and states that only the uredinial stage occurs on peach, while on the plum urediniospores may or may not be present, but there are sometimes a few mingled with the teliospores. Cooper's (14) publication is a repetition of that of Scribner. Pierce (32) amends the statement of Scribner regarding spore forms on the peach, stating that "The spores are mostly uredospores, although the teleutospores are often found at least in California." . . . "In the Annual Report of the Commissioner of Agriculture for 1887, pp. 353–354, it is said that no teleutospores are developed on the peach. Although less abundant than the uredo form, they have invariably been found in badly infected peach leaves in southern California, and these leaves have

been obtained from widely separated points in Los Angeles and Orange counties. They were fully matured by the middle of October." Smith and Smith (36) report the rust on almond, peach, plum, and prune. Barrett (7, 8, 9), who studied the disease in southern California, found a limited production of teliospores on the peach. Blasdale (10) states: "Uredinia and more rarely telia on cultivated peach, plum, prune, almond, and apricot, especially in the southern part of the state, but widely distributed." Many writers elsewhere, particularly in Australia, have noted the comparative scarcity of teliospores of this fungus upon the peach. Cunningham (15) states that in New Zealand teliospores are produced only on the plum. Cobb (13, pp. 232–233) stated that teliospores are usually absent on the peach but noted that, where the latter was in close juxtaposition to rusted plum trees, teliospores were found on both hosts. He suggested that there might be two different species or strains of rust involved in this case. Others have suggested the same possibility. McAlpine (27, p. 25) says: "It is remarkable that the teleutospores are comparatively rare on the apricot and peach and less so on the almond, but the plum seems to have some peculiarity in its constitution which stimulates the formation of the resting spores." Darnell-Smith (16) states that urediniospores and teliospores are found on peach. "As yet only two forms of spores are known for this species of *Puccinia*,—the uredo or summer spore, and the teleuto or winter spore. These spores are produced in varying proportions on different plants. On some hosts the uredospores greatly predominate over the teleutospores, while on other hosts the reverse is true. It appears probably that conditions of food, humidity, climate, and season all tend to vary these results. Both spore forms are produced on the under side of the leaves, and probably both may, under some conditions, serve as winter spores."

Aecial stage. Tranzschel, according to Arthur (3), successfully infected almond, *Amygdalus communis* L., blackthorn, *Prunus spinosa* L., and cherry plum, *P. divaricata* Ledeb., with aeciospores from *Anemone coronaria* L. and *A. ranunculoides* L. (*Aecidium punctatum*, described by Persoon in 1796.) Arthur (3, 4) found aecia on Anemone, Hepatica, and Thalictrum in the eastern United States and inoculated wild black cherry, *P. serotina* Ehrb., wild plum, *P. americana* Marsh, cultivated cherry, *P. cerasus* L., and peach, *P. persica* Sieb. and Zucc., with spores of *Aecidium hepaticum* Schw. from *Hepatica acutiloba*, obtaining infection only upon *P. serotina*. He repeated the inoculation the following year upon *P. serotina* and peach with the same result. He also inoculated peach with urediniospores from *P. serotina* "under seemingly most favorable conditions," but no infection resulted. Jackson (25, pp. 261–283) states that the aecial stage on Anemone has never been found in the West. Blasdale (10):

"Aecia not known from California but found on various species of *Hepatica, Anemone*, and *Thalictrum* in the eastern United States." Brooks (1), in England, successfully inoculated plum leaves with aeciospores from *Anemone coronaria*. Cunningham (15), in New Zealand, says "the aecidial host is an introduced species and is found in but a few localities; yet this rust is prevalent throughout New Zealand on stone fruits." He also states that "as viable uredospores are to be obtained nine months of the year it is probable that this is spread chiefly by these spores." Arthur (5) states: "The aecia are not always required for the propagation of the species, particularly in the warmer regions of its range, where the rust occurs in greatest severity, largely in the uredinial stage." Also: "On the twigs cankers are produced in which uredinia live from one year to the next and perpetuate the rust."

In the district in which this work was done no indication of an aecial stage of the fungus has been found; in fact, none of the probable hosts occur there, except possibly species of Thalictrum in the extensive river bottoms.

SUMMARY

A severe epiphytotic of a rust-fungus disease of peaches occurred in the principal canning-peach section of central California in 1926 and 1927. Serious economic loss was caused by disfiguration of the fruit, which ruined it for canning purposes, as well as by defoliation of the trees.

A description of the disease and the causative fungus is given.

The uredinial stage was the only form of the fungus observed.

Overwintering of the fungus was found to be dependent mainly on twig infections which originate in the fall, remain latent and invisible through the winter, and then develop as uredinial sori in early spring. The fungus also was carried over to a certain extent by overwintering of older sori on the previous season's twigs and by renewed production of urediniospores in spring from such sori.

Subsequent infection of leaves and fruit was found to be dependent upon the presence of sufficient inoculum and the occurrence of periods of several days of rainfall and high humidity.

For the germination of urediniospores a period of at least 3 hours in moisture-saturated atmosphere was found necessary.

The viability of urediniospores attached to living leaves was found limited to a period of about 6 weeks. On detached dried leaves the spores were somewhat shorter-lived.

Urediniospores of the peach-rust fungus germinated over a range of temperature between 8° and 38° C. The optimum temperature was found to lie between 13° and 26° C. The best growth of the germ tubes was observed at about 22° C.

Sulphur and sulphur compounds were found to be more toxic than copper to germinating urediniospores of the fungus. Mineral oil seemed to stimulate germination.

The identity of the fungus with relation to the prune-rust fungus *Tranzschelia punctata* (Pers.) Arth. (*Puccinia pruni-spinosae* Pers.) is discussed. In the present instance no spore form other than the uredinial was found on the peach and the fungus did not attack trees of other species of Prunus (prune, almond, apricot, cherry), even when they were growing immediately adjacent to badly affected peach trees. In the Santa Clara Valley, California, it was observed that the telial stage of the rust usually develops very abundantly on the leaves of prune trees in late fall, but the fungus was not found on adjacent peach trees.

No aecial form of the fungus was observed.

DIVISION OF PLANT PATHOLOGY,
UNIVERSITY OF CALIFORNIA,
BERKELEY, CALIFORNIA.

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THE INFLUENCE OF HYDROGEN-ION CONCENTRATION AND OF SODIUM BICARBONATE AND RELATED SUBSTANCES ON PENICILLIUM ITALICUM AND P. DIGITATUM[1]

RAIMUND H. MARLOTH[2,3]

INTRODUCTION

Plant pathologists have in recent years become dissatisfied with their work in controlling diseases in the mere knowledge that a certain substance is effective in destroying a causative organism and have begun to inquire just how the results are brought about. The new viewpoint calls for a physiological investigation of the organism, particularly in the case of fungi.

The behavior of microorganisms in their relationships to acid and alkali is a phase of the physiology of fungi which has received much attention from investigators since the end of the last century. A complete review of the investigations of the earlier workers on the effect of acid and alkali on microorganisms is given by Webb (23), Johnson (8), and Myers (14). It was not until the measurement of active acidity as apart from titratable acidity and alkalinity was made use of by the plant pathologists that a more exact understanding of the action of the hydrogen and hydroxyl ions on microorganisms was possible; therefore, the results obtained previous to this period can be regarded only as indicative.

The two most important citrus fruit-destroying fungi in California are *Penicillium italicum* Wehmer (blue contact mold) and *P. digitatum* Saccardo (green mold). Dr. H. S. Fawcett, upon examination of five-hundred boxes of stored oranges in four packing houses in central California in 1927, found that of the decayed fruit 52 per cent was due to green mold, 32 per cent due to a mixture of blue and green molds, and 11 per cent due to blue mold, a total of 95 per cent. The rapid development of the citrus industry in California after 1900 resulted in a large increase in the quantity of fruit shipped, and investigations were commenced on the control of various decays that were responsible for considerable loss by the time the

[1] Paper No. 226, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California. Abridgment of thesis submitted in partial satisfaction of the requrements for the degree of Doctor of Philosophy in Plant Pathology in the Graduate Division of the University of California.

[2] Overseas Scholarship Holder, Department of Agriculture, Union of South Africa.

[3] The writer wishes to express his deep appreciation to Dr. H. S. Fawcett for his kindly influence and beneficial suggestions during the performance of this work and to the Director of the University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California, for the courtesies extended to the writer as a visiting graduate student.

fruit had reached the markets, particularly those in the east. At that time the measures adopted were more in the nature of decay deterrents. Woodworth (28) mentioned refrigeration, ventilation for removing excess moisture, and wrapping the fruit to absorb moisture. For packing houses, sanitation was the main feature. Two angles of the cause of decay soon afterwards received attention: (1) the condition of the fruit which allowed infection and (2) the source of decay. Greater care in the handling of the fruit during picking, washing, and packing reduced decay considerably by reducing the number of wounds in the rind, which provide ideal places for natural inoculation. The treatment of the fruit itself was commenced after Smith (21) found that the washing tank was one of the main sources of infection of lemons by the brown-rot organism, *Pythiacystis citrophthora* Sm. and Sm. The addition of either Formalin, permanganate of potash, or copper sulphate to the water in the washing tank was advised. However, this treatment was not satisfactory for the control of blue and green mold on oranges, and, when experimentation with borax, then already a well-known preservative for articles of food, showed that by immersing the fruit in a five per cent solution almost complete control of green mold and some control of blue was obtained, it was not long before packing-house machinery was designed which would permit the commercial use of this process.

In 1927 Mr. A. H. Morgan, an orange grower in Rialto, California, found that the treatment of oranges with sodium bicarbonate gave as good control of the decay due to these molds as did borax or a mixture of borax and boric acid. Also, at about this time, the holders of the patent rights for the use of borax in the prevention of mold decay of citrus fruits, following a favorable court decision, started to enforce their rights to royalties for its use. Since the inventor of the sodium bicarbonate process had taken out a patent and given the free use of his process to the orange growers for ten years, a change was made in most packing houses from borax to bicarbonate when it was found that the latter was successful on a commercial scale. Although the use of sodium bicarbonate is more effective than borax in reducing blue contact mold, it is not so effective against green mold.

Here then is the problem: In just what manner is the prevention of decay effected by these substances, sodium bicarbonate in particular, and are the differences in reaction of two such closely related fungi to the treating substances such as to confirm the observations made in practicing decay prevention? Further, is the action of sodium bicarbonate specific or does it owe its action to some general physiological phenomenon?

MATERIAL AND TECHNIQUE

Since it is well known that strains of a certain fungal species will show differences in physiological behavior, it appeared desirable to conduct the

investigations with material from standard cultures. Hence, cultures were propagated from a single colony. The originals, *Penicillium italicum*, No. 1437, and *P. digitatum*, No. 1438, were obtained from the stock cultures of the Division of Plant Pathology, Citrus Experiment Station, University of California, Riverside. The blue contact mold organism is discussed as *Penicillium glaucum* Link in early papers, Smith (20), Powell (16), where its agency in the decay of citrus fruits is fully considered.

The determination of H-ion concentration was made by means of the quinhydrone electrode.

MEDIA

The first phase of the preliminary investigation was concerned with obtaining a satisfactory synthetic liquid medium on which the two fungi would grow equally well. Using 150 cc. Erlenmeyer flasks, a series was inoculated for each fungus for each of the following media: Czapek's, Duggar's, Richard's, Peptone, Pfeffer's, and Coupin's. The ten flasks per series were adjusted so as to have a hydrogen-ion range of from about pH 2.0 to pH 10.0. The adjustments were made with N/5 HCl and N/20 KOH by means of the method used by Karrer and Webb (9). Observations on growth were made daily. While *Penicillium italicum* grew excellently on Czapek's, Duggar's, Pfeffer's, and Coupin's solutions over a fairly wide range of pH, the behavior of *P. digitatum* was unsatisfactory on all.

Thom (22) states that *Penicillium digitatum* refused to grow on synthetic media containing nitrogen as sodium nitrate. To determine whether the nitrate ion was toxic to this fungus or whether it was merely unable to use it as a source of nitrogen, Duggar's solution was made up in four lots with nitrogen supplied as ammonium sulphate, ammonium nitrate, sodium nitrate, and sodium nitrate plus peptone. It was found that only 2 per cent germination and no growth were obtained with the sodium nitrate solution, as compared with 30 per cent germination and good growth with the other solutions. Considering both these results and those from preliminary culture work, it seems that this species of Penicillium cannot utilize the nitrate ion, while the ammonium ion and peptone are usable as a source of nitrogen with some difficulty.

While *Penicillium italicum* formed an excellent mat in about 8 days on the media just mentioned, it took *P. digitatum* twice as long to form a mat which could be used for comparative studies. Since the latter fungus is specific on citrus fruits and the former fairly cosmopolitan, it was thought that the influence of the various portions of the orange would give a clue to the differential nutrient requirements.

Twenty grams of cane sugar per liter were added to strong water extracts of the oil-bearing tissue (*flavedo*), the white of rind (*albedo*), the

TABLE 1.—*Effect of addition of orange juice and total orange extract to Modified Duggar's Solution on the growth of Penicillium digitatum. Time grown, 8 days*

Amount added per 50 cc. of medium	Initial pH	Orange juice		Orange extract	
		Final pH	Dry weight of mats, in gms. (Average of 3 mats)	Final pH	Dry weight of mats, in gms. (Average of 3 mats)
Checks (10 flasks)	6.7	6.4	No growth except a trace in two flasks		
2 drops	6.7	3.0	.132	3.4	.087
5 drops	6.7	3.2	.136	3.2	.153
10 drops	6.6	2.5	.198	2.7	.219
2 cc.	6.4	2.2	.371	2.6	.350
5 cc.	6.2	2.2	.398	2.3	.376

total rind, the pulp, and the total orange. Mycelial growth of both fungi was poor but sporulation very dense on the first two extracts, while on the remaining three both produced excellent mats and normal sporulation. Whether this marked stimulation in growth of *Penicillium digitatum* was due solely to the organic nitrogen supplied is doubted in view of the results shown in table 1. To determine in greater detail the influence of such substances in citrus fruits on the growth of these fungi on synthetic liquid media, an orange extract was produced by grinding up the whole orange, adding one liter of water to each kilogram of mash, autoclaving at 14 pounds pressure for 30 minutes, then straining through cheesecloth, and again autoclaving for 30 minutes. Orange juice was obtained from fresh fruits with a reamer and then autoclaved. From 2 drops to 10 cubic centimeters of these substances were added to flasks containing 50 cc. of sterilized Duggar's solution by means of pipettes. This work was done in a well-sprayed inoculating chamber. Eight days after inoculation the mats were taken out and the dry weights determined.

No growth was obtained in 8 of the 10 check flasks. The two remaining flasks showed some mat formation. They had received an extra heavy inoculation, tufts of sporulating mycelium having been introduced in addition to the spores as added to the other flasks. The addition of two drops of orange juice, having 1.6 per cent total solids which gave 18 per cent ash, allowed an average growth of 0.132 gm. dry weight. When the amount added was increased to 2 cc. and 5 cc. the increase in weight of mat formed was considerable and may be partly explained by the addition of an extra supply of nutrients. The addition of the ash of the juice failed to give the stimulation observed with the juice itself. No significant difference is

noted between the influence of the orange extract and the orange juice, while similar preparations from grapefruit and lemons in subsequent experiments gave similar results.

Williams (27) has shown that for normal growth baker's yeast was dependent on an adequate supply of a water-soluble vitamin in the medium. Willaman (26) found that a small amount of prune, peach, or apple juice and decoctions from other plant materials added to synthetic media on which *Sclerotinia cinerea* would make no or extremely weak growth allowed the fungus to grow at a normal rate. These decoctions and juices apparently supplied a substance which behaved as a vitamin.

Whether it can be postulated that *Penicillium digitatum* requires for its growth the presence of a vitamin found in citrus fruits, hence explaining its specificity, is problematical. Unfortunately, the continuation of this phase of the nutrition of this fungus lies outside of the scope of the present paper, but repeated experiments confirmed this influence of the orange extracts. As a result of this phase of the work a synthetic liquid medium of which the proportions of the principal nutrient constituents were known and which allowed approximately the same rate of development of excellent mats of both fungi was now available.

The writer has decided to name the synthetic medium used "Modified Duggar's Solution." The modifications include the use of ammonium sulphate since the nitrate ion has been shown to be unusable by *Penicillium digitatum*, the monohydrogen phosphate because its use results in a pH value of the solution nearer the neutral point, and a reduction in the amount of magnesium sulphate to lessen the amount of precipitate formed in alkaline solutions. The chemicals used were the usual C.P. salts, and these contain impurities in sufficient quantities to supply all the essential elements for fungal growth.

Modified Duggar's Solution:

$(NH_4)_2SO_4$ 10 gms.; K_2HPO_4 5 gms.; $MgSO_4 \cdot 7H_2O$ 1 gm.; $FeSO_4$ trace; cane sugar 25 gms.; H_2O 1 liter.

From 2 to 5 per cent orange extract was added to the medium at time of making, the percentage depending on the amount available.

Orange-glucose-potato agar was found to give a more vigorous growth for both fungi, with plentiful sporulation, and was used throughout the work. Five per cent of sterilized orange extract was added when filling the test tubes for slants, for it was found that if the orange juice was autoclaved with the agar the latter would not harden. This process was performed in the inoculating chamber, the test tubes and plugs having been sterilized before filling, and a re-autoclaving after filling was not necessary.

Cultures. The liquid medium was made up in large enough quantities for each complete experiment. Each flask contained 50 cc. of the medium,

Erlenmeyer flasks of 150 cc. capacity being used throughout. Spores from slants were transferred to the culture flasks by means of a chromel wire loop. It was found that spores from cultures up to three months old did not vary in percentage of germination, and as long as an even distribution of spores over the surface of the medium was obtained the final mat weight was not influenced by the inoculation process.

The age of the culture of a fungus at which the mat attains maximum weight varies mainly with the medium and the temperature at which grown. The influence of temperature on growth was minimized by setting up a complete experiment at once, the flasks being kept on trays at room temperature. Fawcett and Barger (6) showed that the two fungi concerned here were generally affected similarly by different temperatures. To determine the approximate length of time cultures should be grown to obtain best results before autolysis sets in, a series of flasks for *Penicillium italicum* and *P. digitatum* was placed in the incubator at 21.5° C. Four flasks were removed daily, the mats washed in water, dried in the oven for 24 hours at 95° to 98° C. and weighed. The growth curves for the two fungi are almost similar, with *P. italicum* reaching maximum weight in 6 days and *P. digitatum* in 7 days; autolysis is slow up to 12 days (Fig. 1). Since conditions

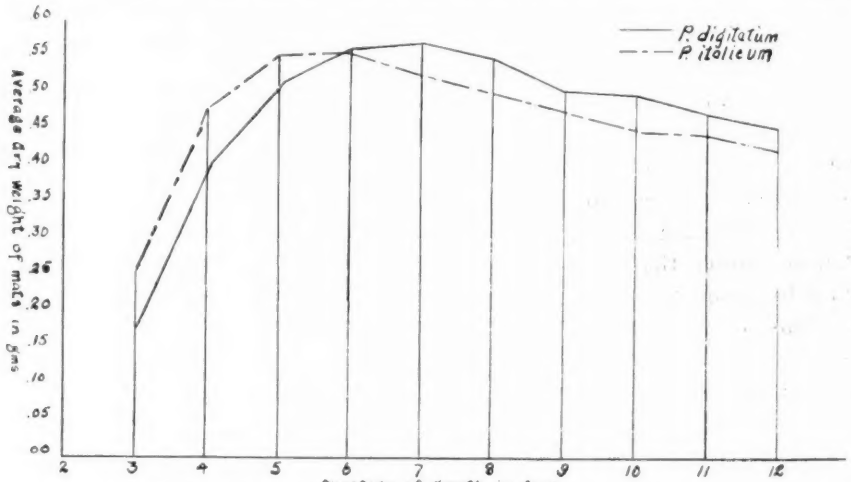

FIG. 1.—Mat growth per day of *Penicillium italicum* and *P. digitatum* at 21.5° C. In modified Duggar's solution + 10 per cent orange extract + 1 per cent potassium citrate (50 ml. per flask).

here were almost optimum for mat growth, it was decided to grow all mats for 8 days in future experiments to allow for lagging in growth due to any slightly unfavorable conditions. The final hydrogen-ion concentrations of media were always determined.

Germination. For the germination experiments Van Tieghem cells were sealed with paraffin to glass slides, two cells to each slide. The bottom of the cell was covered with distilled water and a touch of vaseline put at two opposite spots on the upper edge of the ring, upon which the cover glass rested. With germination experiments in different media, suspensions of spores, obtained from slant cultures about one month old, were made. A drop of each suspension was then placed on a cover glass and inverted over its respective cell. This was done in duplicate for all determinations. When germination had proceeded the desired length of time, the cover glasses were raised and a few drops of chloroform introduced into the bottom of the cells. This was done to stop germination and growth during periods of observation and measurement.

Where the spores were subjected to different treatments by bringing them in contact with various alkali solutions, a slight modification of the technique of Henderson Smith (7) was used. Spores were introduced into 15 cc. of the treating substance in a test tube, well shaken, and 1 cc. of the suspension run into 10 cc. of distilled water in a centrifuge tube at the exact completion of the time of treatment, the time being noted on a stop watch. This was then centrifuged for 10 minutes, the water poured off and a few cc. of Modified Duggar's Solution plus orange extract squirted into the tube, making a new suspension from which hanging-drop cultures were made.

Germinated spores which were to be subjected to different treatments were obtained by dusting a very thin film of spores on Modified Duggar's Solution (in further references to Modified Duggar's Solution it will be understood that 5 per cent orange extract has been added unless otherwise stated), just covering the bottom of a large flat specimen dish. When examination showed that germination was well under way, the germ tubes not being too long, the medium was poured out and a suspension made. This suspension was then added to the treating substances, the amount being determined by the heaviness of the suspension, and the process was continued as described above. Observations on the amount of germination were made on both cells on each slide, three fields in each cell generally being counted. Careful counts were made in all cases where the germination was less than 20 per cent, for it was in this range that results that would count were looked for.

HYDROGEN-ION-CONCENTRATION STUDIES

In obtaining the suitable medium already mentioned, it was noted that the pH of the media after supporting growth showed great variation both within each series and between different media, but that the results produced by the two fungi were somewhat similar. The change in pH from

the initial value seemed to have a relation to the amount of growth. Klotz (10) showed that *Aspergillus niger* began autolyzing after 3 days, the disappearance of the carbohydrates from the culture medium being synchronous with the beginning of autolysis. By growing *Penicillium italicum* on Czapek's solution, the writer found that this fungus autolyzed extremely rapidly after the ninth day where the initial hydrogen-ion concentration of the medium was pH 4.0. The medium showed a marked change in pH towards the alkaline side only after autolysis had set in. That *P. italicum*

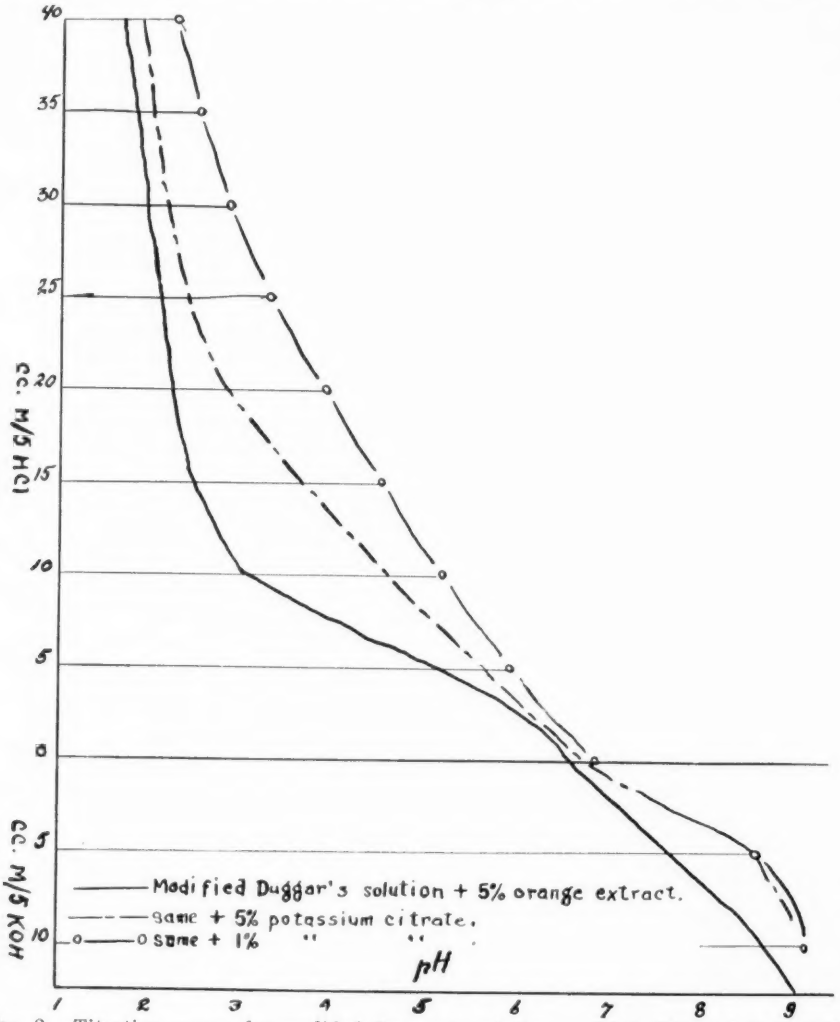

FIG. 2.—Titration curves for modified Duggar's solution. (M/5 HCl and M/5 KOH titrated into 50 ml. medium. Determinations by quinhydrone electrode.)

produces oxalic acid when grown on media containing sucrose has been shown by Currie and Thom (5), the production being considerable when calcium carbonate is present to remove the acid in combination when formed. Since the two fungi here studied changed the reaction of the medium similarly, it is accepted that *P. digitatum* also has oxalic acid as one of the main products of metabolism.

The buffer systems of the medium will largely determine the final pH if the amount of growth is the same at different initial pH values. This change in reaction of the medium invalidates experiments to find the optimum hydrogen-ion concentration for growth where only the initial values are known; the medium has to be kept at constant pH for true results. To be able to interpret comparatively the changes in reactions of the media due to the fungus growth, electrometric titrations of Modified Duggar's Solution with and without potassium citrate were made. The titration curves are shown in figure 2. The addition of citrate allows fungi to be grown over a wider range of hydrogen-ion concentrations of the media without the differential change in the reaction influencing final results. The direct and indirect influence of the citrate on the growth of the fungi was tested out in a comprehensive experiment in which the concentrations of citrate and sucrose in the medium were varied. The results are shown graphically in figure 3. It is seen that neither fungus can utilize the citrate as a source of carbon for metabolism when no sucrose is present. Camp (3) found that citrate mixtures adjusted to a favorable pH value proved to be efficient as a supplementary carbon source when used with small quantities of dextrose for all the fungi he worked with except *Penicillium digitatum* and *Phomopsis citri*. Sakamura (18) holds that the favorable influence on the growth of *Aspergillus niger* by the addition of oxalate, citrate, or phosphate of potassium is due to its helping to maintain a favorable pH in the media. The increase in the amount of sucrose when no citrate was present resulted in almost proportional increase in mat growth in the results recorded here, but the mat growth in the medium containing 20 gms. sucrose and 10 gms. citrate is greater than that in which 30 gms. sucrose is present. Whether this extra growth is due to the ability of the fungus to utilize the citrate or whether it was because the medium maintained a hydrogen-ion concentration most favorable for growth cannot be stated, but the writer thinks it is mainly because a favorable reaction is maintained in the medium.

The growth of fungi in a medium of constant hydrogen-ion concentration was attempted by Sideris (19), but the nearest approach to such conditions has been attained by Marloth (13) with his apparatus which makes possible the repeated renewal of the medium under the fungus mat. An improvement on that apparatus is offered in figure 4. The tube G has been

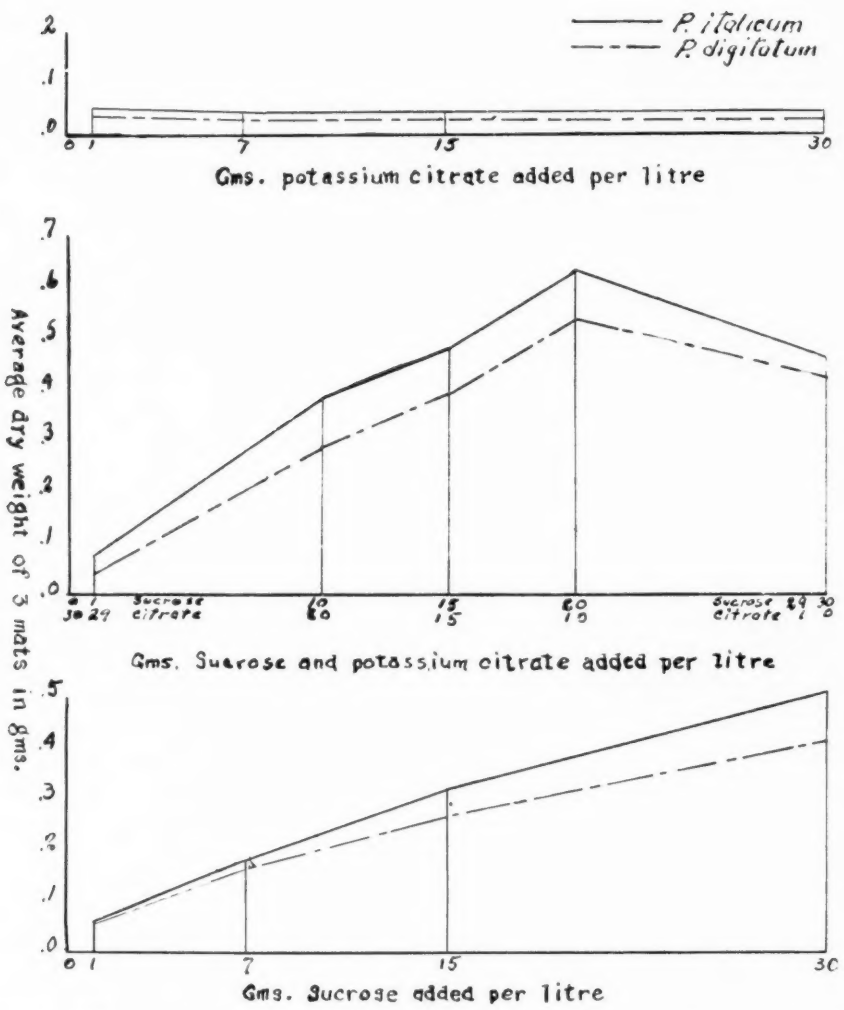
P. italicum
P. digitatum
Gms. potassium citrate added per litre
Average dry weight of 3 mats in gms.
Gms. Sucrose and potassium citrate added per litre
Gms. Sucrose added per litre

FIG. 3.—Effect on growth of varying the proportions of sucrose and citrate in the medium. (Modified Duggar's solution + 2 per cent orange extract.)

extended to the bottom of the flask to prevent the new supply of medium lodging on top of the mat. Furthermore, round-side flasks are more satisfactory than Erlenmeyer flasks.

Using the improved apparatus with 70 cc. Modified Duggar's Solution in each flask, cultures were grown as follows:

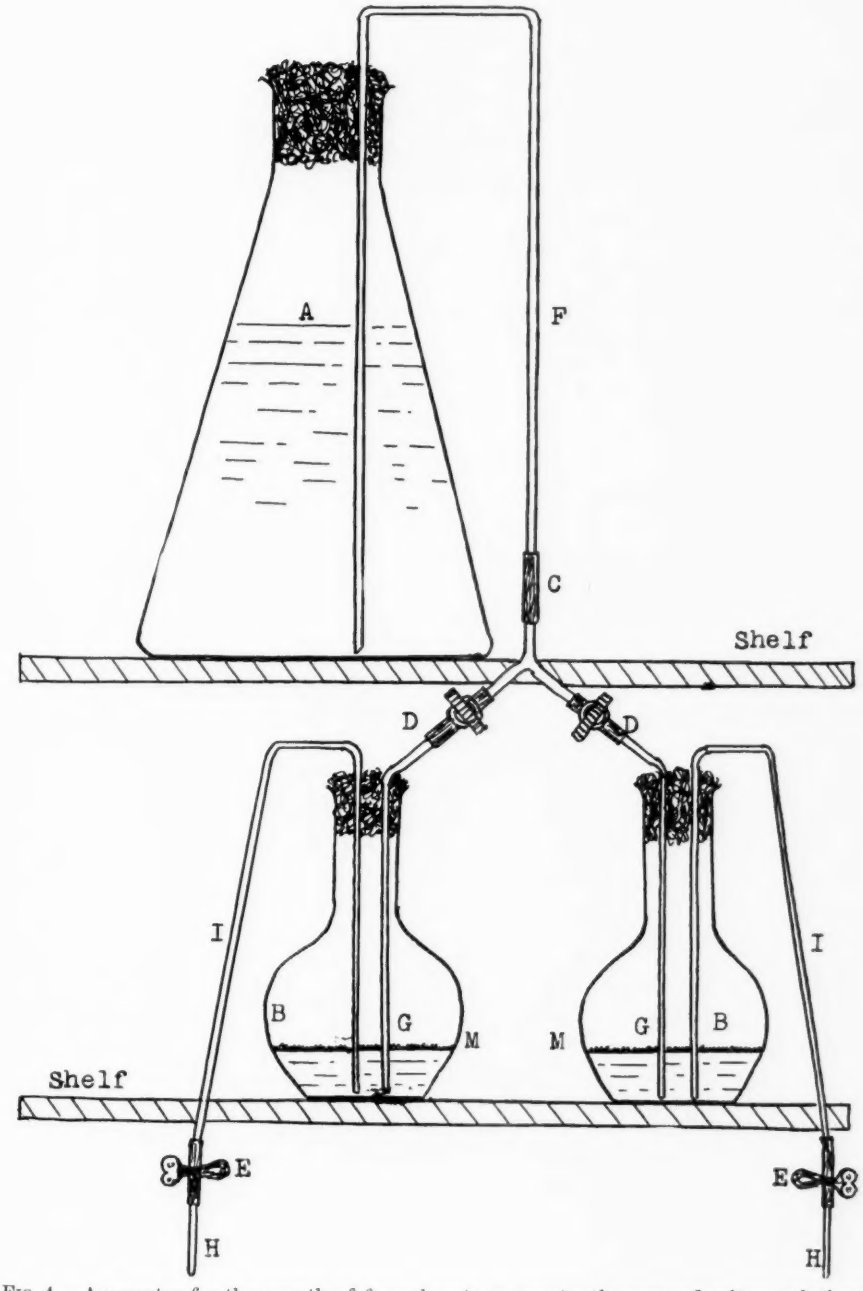

FIG. 4.—Apparatus for the growth of fungal mats on constantly renewed culture solutions.

No citrate					
P. italicum, 8 days					
Initial pH	2.05	2.5	3.9	4.6	6.0
Av. dry wt. of 2 mats, in gms.	.139	1.058	1.265	1.663	1.383
P. digitatum, 9½ days					
Initial pH	2.3	2.7	4.9	5.9	6.5
Av. dry wt. of 2 mats, in gms.	.032	.822	.818	.667	.376
1 per cent potassium citrate					
P. italicum, 8 days					
Initial pH	2.5	3.15	5.6	6.05	6.8
Av. dry wt. of 2 mats, in gms.	1.160	2.947	2.357	2.049	1.631
P. digitatum, 8 days					
Initial pH	2.5	3.0	3.7	5.0	6.75
Av. dry wt. of 2 mats, in gms.	.137	1.898	1.935	1.699	.394

The "no citrate" and the "citrate" are not comparable, due to the former having been grown during cold weather and the latter during very warm weather. Further, in the case of the no citrate series, the daily drop in the pH of the medium was relatively large, from pH 5.6 to 4.1 for *Penicillium italicum* and from pH 5.8 to 4.3 for *P. digitatum* after the mats were well formed. With the citrate series the daily change in reaction was smaller, the greatest being from pH 5.6 to 4.8 for *P. italicum* and from pH 6.7 to 5.9 for *P. digitatum*. These marked changes in reaction usually occurred with media having an initial pH above 5.0, whereas the change in media with pH 2.5 was very slight. Table 2 shows the daily drop in pH value due to the growth of *P. digitatum* on medium plus citrate. Check flasks grown on 70 cc. of medium for 8 days gave a final pH of 2.1 for *P. italicum* and 2.45 for *P. digitatum* when no citrate was present as a buffer. No cultures have been found to have a lower final pH than 1.9 and 2.2, respectively, for these two fungi. This final hydrogen-ion concentration of the media depends somewhat on the initial pH, for when no citrate was present as a buffer the reduction was from 2.7 to 2.0, 4.4 to 2.5, and 6.4 to 2.6 for *P. italicum* and from 2.7 to 2.2, 4.4 to 2.4, and 6.4 to 2.5 for *P. digitatum* (Table 3).

The limits of the hydrogen-ion concentration of the media which would allow growth of the fungi under investigation were not exactly determined, but it is seen that they have a fairly wide optimum range. Numerous cultures have indicated that *Penicillium italicum* will not form a mat if the initial pH of the medium is below 2.1 or above 7.9, and *P. digitatum* not below 2.4 or above 7.8. Particularly in the case of the latter fungus the mats are very unsatisfactory above the neutral point, being generally submerged. However, a fair amount of mycelium will develop within the liquid medium up to pH 8.5.

TABLE 2.—*Growth of Penicillium digitatum in flasks. Medium renewed daily, with 70 cc. Modified Duggar's Sol. + 5 per cent orange extract + 1 per cent potassium citrate; also daily increase of hydrogen-ion concentration of medium*

Initial pH of medium	Daily pH of renewed solutions in flasks							Final pH in reservoir	Dry weight of mats, in gms.	
	Days								Duplicate flasks	Average
	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth			
6.75	6.45	6.25	6.15	6.1	6.0	5.95	5.9	6.7	.376 .412	.394
5.0	4.9	4.7	4.7	4.65	4.7	4.75	4.8	5.25	1.722 1.676	1.699
3.7	3.65	3.5	3.4	3.5	3.5	3.55	3.55	3.9	1.945 1.722	1.985
3.45	3.2	3.1	3.0	3.0	3.0	3.15	3.2	3.4	1.907 1.788	1.848
2.95	2.9	2.8	2.65	2.6	2.6	2.55	2.55	3.0	1.836 1.960	1.898
2.5	2.5	2.5	2.45	2.45	2.4	2.4	2.4	2.5	.152 .122	.137

TABLE 3.—*Mat growth per day in flasks containing Modified Duggar's Solution + 5 per cent orange extract*

Days of growth	Initial pH 2.7			Initial pH 4.4			Initial pH 6.4		
	Final pH	Av. dry wt. of 2 mats, in gms.	Change in dry wt.	Final pH	Av. dry wt. of 2 mats, in gms.	Change in dry wt.	Final pH	Av. dry wt. of 2 mats, in gms.	Change in dry wt.
Penicillium italicum									
3	2.65	.....	.....	3.1	.158	+.158	4.0	.088	+.088
4	2.4	.133	+.133	2.7	.272	+.114	3.4	.193	+.105
5	2.25	.268	+.135	2.55	.362	+.090	2.8	.337	+.144
6	2.15	.369	+.101	2.5	.403	+.051	2.65	.439	+.102
7	2.05	.423	+.054	2.5	.412	+.009	2.6	.458	+.021
8	2.0	.489	+.066	2.5	.429	+.017	2.6	.461	+.003
10	2.0	.523	+.034	2.65	.408	−.021	2.8	.439	−.022
12	2.05	.476	−.047	2.8	.376	−.032	2.85	.430	−.009
Penicillium digitatum									
3	2.65	.....	.....	2.7	.252	+.252	4.45	.097	+.097
4	2.5	.038	+.038	2.5	.406	+.154	3.3	.211	+.114
5	2.4	.099	+.061	2.4	.472	+.066	2.8	.348	+.137
6	2.35	.155	+.056	2.45	.493	+.021	2.55	.475	+.127
7	2.25	.228	+.073	2.4	.488	−.005	2.55	.472	−.003
8	2.25	.274	+.046	2.45	.485	−.003	2.6	.446	−.026
10	2.2	.359	+.085	2.55	.423	−.062	2.65	.430	−.016
12	2.2	.360	+.001	2.6	.410	−.013	2.9	.414	−.016

The morphological nature of the mats of both of these fungi was directly influenced by reaction of the media, those grown on media of pH 2.6 and below being brittle and wrinkled with no or only slight sporulation, while they were slimy with excellent sporulation on media of pH 6.0 or above.

Two series of cultures of 64 flasks each were grown, one with citrate and the other with no citrate in the medium, three initial pH values of 2.7, 4.4, and 6.4 being used for each series. Two flasks were removed daily, the final pH determined, and the mats dried and weighed. The results as shown in table 3 bring out two points already noted; namely, that *Penicillium digitatum* grows more rapidly than *P. italicum* and that the addition of citrate to the medium results in better growth for both fungi. The three initial pH values of the media were chosen with 4.4 as the optimum for growth and 2.7 and 6.4 as being near the acid and alkaline limits. The wider range of tolerance for hydrogen-ion concentration is shown by *P. italicum*. Further, this fungus reduced the pH value of the medium to 2.0 and *P. digitatum* to 2.2 in 8 days, and these figures may be taken as the lower limit for growth, the latter fungus not having so low a limit as *P. italicum*.

Whether the maximum hydrogen-ion concentration of a medium in which growth will start or which will allow growth to continue should be regarded as the lower limit is debatable, but nevertheless it remains that *Penicillium italicum* is more tolerant towards acid for growth than *P. digitatum*. This order of tolerance remains true also on the alkaline side, for abnormal and restricted growth occurs with *P. digitatum* at a pH value where *P. italicum* forms almost normal mats.

Germination. The germination of spores of fungi in relation to the hydrogen-ion concentration of the media has received excellent study by Webb (23, 24). In his first paper he records that the maximum germination for most fungi studied was between pH 2.8 and 3.1. The highest reaction which allowed germination, although slight, was pH 10.0 at 27° C., *Penicillium cyclopium* being the fungus. The second paper records the limits and optimum range of germination for certain fungi on different media at various temperatures. He found that *P. italicum* had the following ranges: Mannite: Ortho-phosphoric acid 2.0 to 7.7, Czapek 1.8 to 8.2, peptone 1.6 to 7.2, beet decoction 2.4 to 8.9. The optimum range was pH 3.0 to 4.0 for all media.

Webb summarizes as follows: "On comparing equal concentrations of H and OH ions, the OH ions appear to be relatively more toxic to spores studied than H ions. The toxicity of H ions is fairly independent of the other conditions studied, while that of OH ions tends to be more or less variable or antagonizable, according to the composition of the medium."

TABLE 4.—*Effect of pH of medium on germination of spores in Van Tieghem cells. Modified Duggar's Sol. + 5 per cent orange extract. No citrate*

pH	Percentage of germination			
	Penicillium italicum		*Penicillium digitatum*	
	24 hrs.	60 hrs.	24 hrs.	60 hrs.
1.5	0	0	0	0
1.95	0	15	0	0
2.4	25	75	10	40
3.0	80	95	70	80
3.7	90	E	80	90
5.3	90	E	80	E
6.1	90	E	70	E
6.5	90	E	70	E
7.05	60	E	50	E
7.1	50	E	60	E
7.8	40	E	60	E
8.2	40	E	50	E
8.4	60	E	70	E
9.0	50	E	40	E

E = Hanging drop overgrown, thus unable to count spores.

TABLE 5.—*Effect of pH of medium on the germination of mature spores in Van Tieghem cells. Modified Duggar's Sol. + 5 per cent orange extract. No citrate. Set up in duplicate. Temperature 24°–30° C.*

pH of hanging drop	Percentage of germination in 19 hours			
	Penicillium italicum		*Penicillium digitatum*	
6.55	100		45	
7.85	98		40	
8.1	99		32	
8.2	98		45	50
8.4	98		42	50
8.6	97		50	
8.9	96		31	20
9.2	98		52	60
9.4	62	85	37	57

The wider range of tolerance for H ions exhibited by *Penicillium italicum* in comparison with *P. digitatum* for growth finds a parallel in the germination experiments recorded in tables 4 and 5, especially on the acid side. Of the media used, Modified Duggar's Solution allowed maximum germination. A series of cells was set up with the medium used for the

hanging drops adjusted to 14 different pH values. The resulting percentage of germination after 24 and 60 hours is presented in table 4. Here the lower limit for *P. italicum* is 1.95 and 2.4 for *P. digitatum*. A small subsequent similar experiment gave 3 per cent germination at pH 1.85 and 2 per cent at pH 2.75, respectively, for the two fungi, and these values are presented as the lower limits of germination on this medium. The amounts of germination recorded on the alkaline side are subject to criticism, for, while the initial pH of the hanging drop was as is given in the table, reference to figure 2, which gives the titration curve for the medium, will show how weak the buffer system is in that range. This explains why media adjusted to pH values on the alkaline side change towards the neutral point, even when merely standing in cotton-stoppered flasks, independent of the change in reaction of the hanging drop due to the first slight growth of the spores. A repeat experiment is recorded in table 5. In this the period for germination was shortened and the cover glasses were sealed to the rings to exclude the entrance of a constant supply of carbon dioxide from the air. In addition, the pH was determined for flasks of the adjusted media before and after the experiment and the lower value given as the pH of the hanging drop. A retardation of germination is noted above pH 9.2 for *P. italicum*, but the behavior of *P. digitatum* was very variable. As it was not possible to adjust Modified Duggar's Solution to pH values higher than 9.6 without the addition of so much alkali as to radically alter the composition of the medium, the upper limit of germination for these fungi on this medium has not been determined, although it was found that *P. italicum* gave only 20 per cent germination and *P. digitatum* 10 per cent at pH 9.7. Using Sörensen's glycocoll buffer mixture, as given in Clark (4), no germination for either fungus was obtained above pH 9.7. No germination of the spores of either of the blue or green molds was obtained in 2.6 and 10 per cent solutions of sodium and potassium carbonate and bicarbonate and 4 per cent sodium borate in water to which 2 per cent sucrose had been added. The pH values of the bicarbonate solutions were 8.3 to 8.6, of the carbonates, 11 to 11.4, and of the borate, 9.4. An interesting observation in the preliminaries to this experiment was that the percentage of germination of both fungi was twice as great in sucrose dissolved in tap water as in sucrose in distilled water and much less when dextrose was used instead of sucrose, the highest percentage of germination in any of these media being only 40 per cent. A further determination on the lower limit of germination was made, this time with KCl–HCl buffer solution plus 2 per cent dextrose. Again it was noted that *P. italicum* has a lower limit, pH 2.0 as against 2.2 for *P. digitatum*, the latter being relatively inhibited below 2.4.

INFLUENCE OF THE SODIUM ION

In studying the toxic properties of a chemical substance it is desirable to know the effect of the individual ions as well as that of the combined compounds on the microorganisms. With mercury salts it is known that their toxic properties lie in the mercury ion, as shown by Madsen and Nyman (12). Working with *Penicillium glaucum* (probably *P. italicum*) Boeseken and Waterman (2) concluded that the cause of the toxic action of certain metallic salts, as well as boric acid, is not purely physical, but may be primarily chemical; the most active elements form complex compounds within the organism. With sodium bicarbonate it is not even known whether the toxic property is an indirect or a direct one, that is, whether it lies in the whole or part of the compound or in the reaction caused by its dissolution in water. Levine, Buchanan, and Toulouse (11) showed that the addition of NaCl, Na_2CO_3, or Na_3PO_4 to NaOH markedly decreased the killing times for bacteria, suggesting that it is the undissociated NaOH which, penetrating the cell, causes death. That this is controversial is shown by Meyers (14) who states "that the disinfectant action of an alkali is one largely due to the concentration of free hydroxyl ions in solution has been generally accepted." He offers a review of the work of other investigators, most of which pertained to bacteria, and the alkalies used were strongly dissociating ones, such as hydroxides and carbonates. With sodium and potassium bicarbonate the reaction of a 6 per cent solution is only pH 8.5. Since it has been shown earlier that spores of *P. italicum* and *P. digitatum* would germinate in media of pH 9.7, the disinfectant property of sodium bicarbonate cannot wholly be explained by the hydroxyl-ion concentration of the solution.

To test the influence of the sodium ion on the growth and germination of the Penicillium species here under investigation, sodium citrate, potassium citrate, sodium chloride, and potassium chloride were added to Modified Duggar's Solution in varying concentrations, calculated in parts per million of sodium or potassium ions. Two series of cultures were grown with each salt, and the growth results are plotted in figures 5 and 6. The data were obtained from 150 cultures in which the cation concentrations of sodium and potassium citrate were from 2,500 to 20,000 p.p.m. Three flasks were used for each value. It was found that potassium citrate up to a concentration of 35,000 p.p.m. of potassium did not inhibit growth and germination of *P. italicum* and that *P. digitatum* was only slightly inhibited relative to the effects of the sodium salt. (References to "inhibition of growth and germination" mean fairly marked inhibition.) Sodium citrate has a marked inhibitory influence on the germination of *P. italicum* at 20,000 p.p.m. and on *P. digitatum* at 15,000 p.p.m. It inhibited the

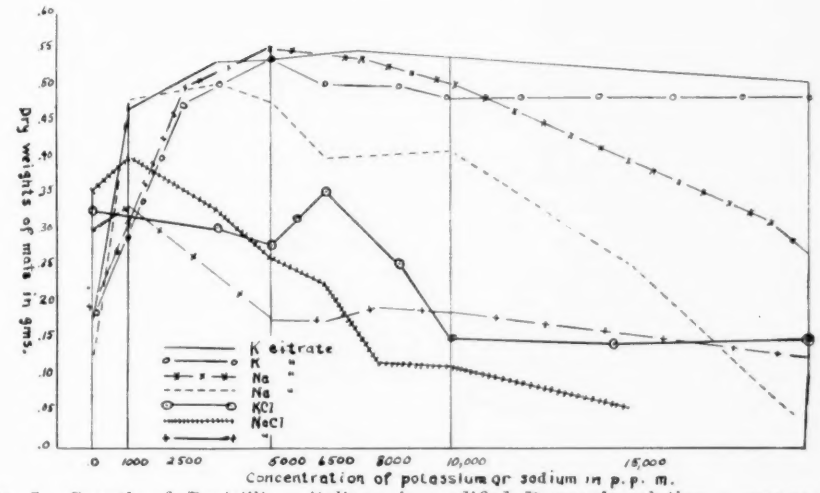

FIG. 5.—Growth of *Penicillium italicum* in modified Duggar's solution + orange extract + varying concentrations of potassium and sodium.

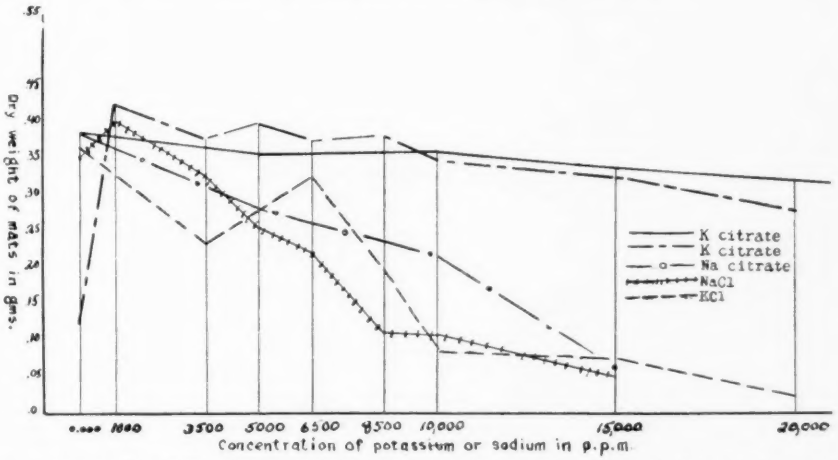

FIG. 6.—Growth of *Penicillium digitatum* in modified Duggar's solution + orange extract + varying concentrations of potassium and sodium.

growth of the latter fungus at 10,000 as compared with 20,000 p.p.m. for the former. Sodium and potassium chloride were similar in their inhibitory effects on the growth of both fungi, the dry weights of mats formed in the presence of 10,000 p.p.m. of sodium or potassium being negligible. The indications are that with these two salts it is the chlorine ion which causes inhibition of growth, especially as the potassium salt does not inhibit germination of *P. italicum* at all up to 20,000 and *P. digitatum* only above 15,000 p.p.m. Unfortunately, the amount of dissociated anions and

cations in the solutions used by the present method could not be calculated, so that the comparison of the toxic influence of the chloride and citrate salts based on the total p.p.m. of cations added is not quite justifiable. Throughout all of these series a favorable initial pH of the media was maintained and the final pH values in no case were beyond the usual limits. That the factor of the osmotic pressure of the solutions had no obvious influence on the results obtained is shown by the normal germination and growth when the medium contained 50 per cent sucrose with an osmotic pressure of 38 atmospheres as compared with 17.3 atmospheres for the solution containing 35,000 p.p.m. potassium as citrate.

In interpreting the recorded results the difference between the sodium and potassium ions seems to warrant the conclusion that the potassium ion up to the high concentrations used exerts no inhibitory action, while the sodium ion, whether added as citrate or chloride, is undoubtedly toxic to both fungi at the higher concentrations but to a greater extent to *Penicillium digitatum*.

EFFECT OF BICARBONATES AND CARBONATES ON SPORES

The critical pH values of the medium for growth of *Penicillium italicum* were found by Johnson (8) to be 1.9 to 2.2 and 9.1 to 9.3 on Czapek's medium, and the toxicity of salts, molecule for molecule, as follows: $KCl < NaCl < Na_2CO_3 < K_2CO_3$. Since the two latter salts give pH values of 10+ when in solution in the concentrations used, the major part of their toxicity is undoubtedly due to the high hydroxyl-ion concentration and to the bicarbonate ion, as will be shown later, when the pH of the medium is 8.2 or slightly higher after the addition of the salts. Pratt (17) shows that 5.6 per cent potassium bicarbonate in Richard's solution, giving a pH value of 8.4, allowed no germination of *Penicillium* sp. spores.

The percentage of the solution of sodium bicarbonate in which the citrus fruits are immersed for treatment is from 3 to 4 per cent, this having been found to be the most suitable concentration for use in commercial packing houses. While it has been found that better control is obtained with a 6 per cent solution it is not practical to use due to the heavy deposits of salt forming on the machinery which conveys the fruit. A 1 per cent solution of sodium carbonate causes injury to the fruit, and this concentration is not sufficient to give such good prevention of decay as the 3 per cent solution. The process used in packing houses is that after being washed in a soap solution with brushes the fruit is immersed in the bicarbonate solution heated to 100° F. for from 4 to 6 minutes. As the fruit comes out of the last tank a thin spray of water washes off the excess treating solution, after which it is dried by means of warm air and then wrapped. Barger (1) treated large quantities of experimentally injured, inoculated, and orchard-

run fruit in baths of sodium borate and sodium bicarbonate to which mixed spores of both fungi had been added. His results of 4 minutes soaking in solutions at 100° F. include the following amounts of decay after 6 weeks.

Injured Fruit

Solution:	Water	4½% sodium borate	3% sodium bicarbonate	5% sodium bicarbonate
Percentage of decay:	86.0	69.3	35.3	32.0

Noninjured Fruit

Solution:	Water	4–5% sodium borate	4–6% sodium bicarbonate
Percentage of decay:	28.3	18.2	16.7

Effect on nongerminated spores. Naturally one of the first questions to arise is whether the spores themselves have been killed or whether the fruit has been conditioned in some way so as to render the start of decay difficult. The effect on the germination of the spores of *Penicillium italicum* and *P. digitatum* of various concentrations of sodium and potassium carbonate and bicarbonate is given in tables 6 and 7, the former re-

TABLE 6.—*Effect of treatment of nongerminated young, mature spores. Temperature 25°–30° C.*

Treatment	Time, in min.	Per cent germination in 24 hours	
		Penicillium italicum	*Penicillium digitatum*
Check	.....	100	80
4% $Na_2B_4O_7 \cdot 10H_2O$	5	86	40
2% $NaHCO_3$	2	97	55
	5	91	50
6% $NaHCO_3$	2	72	15
	5	63	11
2% Na_2CO_3	2	85	90
	5	80	80
6% Na_2CO_3	2	78	50
	5	75	15
2% $KHCO_3$	2	95	90
	5	80	60
6% $KHCO_3$	2	80	60
	5	65	50
2% K_2CO_3	2	98	35
	5	80	25
6% K_2CO_3	2	95	25
	5	65	18

TABLE 7.—*Effect of treatment on nongerminated spores 6 days old. Temperature 20°–25° C.*

Treatment	Time, in min.	Per cent germination in 24 hours	
		Penicillium italicum	*Penicillium digitatum*
Check		95	50
4% $Na_2B_4O_7 \cdot 10H_2O$	10	50	45
	2	95	45
	5	90	40
10% $NaHCO_3$	10	85	35
	25	25	20
	45	20	20
10% Na_2CO_3	2	90	40
	5	80	30
6% $KHCO_3$	2	95	45
	5	90	40
15% $KHCO_3$	10	85	40
	25	70	15
25% $KHCO_3$	10	25	10
	25	10	5
4: 1000 $HgCl_2$	5	0	0

cording results with young mature spores and the latter with spores 60 days old. The success of the experiments depends on the wetting of the spores so as to allow the treating substances to act on the spore wall or the protoplasm within. That this was accomplished is shown by the fact that the mercuric chloride-treated spores gave no germination, showing complete wetting. Since this was an aqueous solution of higher surface tension than the alkali solutions, it may be presumed that the latter completely wetted the spores. Following the two-minute treatment of all concentrations of the bicarbonates and carbonates the reduction in germination of the young mature spores of *P. italicum* is not great enough to be really significant, while with those of *P. digitatum* it is marked. However, a 4 per cent solution of sodium borate is more effective against the latter fungus than the former, this being the case in commercial treatments. Certain facts regarding both fungi are clearly indicated. The resistance of 60-day-old spores against the treating substances as compared with 14-day-old spores is quite marked for *P. digitatum*. The increase in concentration of the treating substances increases the extent of spore mortality. This is without any significant increase in pH value of the solutions, as shown by the values given in table 8. Increase in time of treatment increases the number of

TABLE 8.—*Effect of treating germinated spores. Temperature 22°–29° C. Percentage germinated before treating, Penicillium italicum 70, Penicillium digitatum 40*

Treatment	pH	Time, in min.	Per cent germination in 22 hours					
			Penicillium italicum			*Penicillium digitatum*		
			Total	Growth	Killed	Total	Growth	Killed
Check	7.0	.....	98	E	.....	98	E	.....
4% $Na_2B_4O_7 \cdot 10H_2O$		5	90	G	20	45	F	40
2% $NaHCO_3$	8.3	2	95	E	3	85	E	1
		5	95	G	10	85	E	3
6% $NaHCO_3$	8.4	2	90	F	10	75	E	5
		5	85	F	25	80	V.G.	10
		10	75	P	50	80	V.G.	30
10% $NaHCO_3$	8.5	2	90	G	15	55	F	30
		5	80	F	40	50	F	35
		10	75	P	60	50	P	35
2% Na_2CO_3	10+	2	85	G	15	60	G	15
		5	80	F	25	55	F	20
6% Na_2CO_3	10+	2	80	F	20	50	F	20
		5	70	P	40	45	P	30
10% Na_2CO_3	10+	2	80	F	15	45	P	40
		5	75	V.P.	50	45	V.P.	40
		10	70	V.P.	65	40	None	40
2% $KHCO_3$	8.4	2	95	G	2	80	V.G.	3
		5	90	G	10	75	G	8
6% $KHCO_3$	8.5	2	90	G	10	60	F	15
		5	80	V.P.	50	45	P	35
2% K_2CO_3	10+	2	80	P	10	50	F	15
		5	75	P	25	45	F	20
6% K_2CO_3	10+	2	75	P	15	50	P	25
		5	70	V.P.	50	40	None	40
4:1000 $HgCl_2$	3.0	5	70	None	70	40	None	40

E = Excellent; G = Good; F = Fair; P = Poor; V.P. = Germ tubes not more than 20 μ in length.

spores killed. The sodium salts are slightly stronger disinfecting agents than the potassium salts. The same percentage of carbonate is more effective than the bicarbonate in killing the spores. Nevertheless, the reduction in germination, with the exception of 25 per cent potassium bicarbonate solution for 25 minutes, is not sufficient to explain the degree of prevention of decay obtained in the commercial process. It is true that after the spores have been in the treating tank of sodium bicarbonate for several

hours all will have been killed, but there are sufficient spores in the atmosphere of the packing houses settling on the fruit as it passes along the conveyers after treatment to nullify the results of treatment unless some other factor or factors were involved. The writer believes that he has found this factor to be the thin film of sodium bicarbonate left on the rind of the fruit after drying.

Effect on germinated spores. In the experiments with germinated spores, as mentioned previously, a dusted film of spores was allowed to germinate on liquid medium. Due to the great difference in the rate of germination in such a film, the suspension had to be made when only 70 per cent of the spores of *Penicillium italicum* and 40 per cent of *P. digitatum* had germinated, for otherwise the germ tubes which appeared first would have increased to such a length as to render the making of the suspension and subsequent treatment impossible without breaking off these tubes. Checks of the original suspension were set up at the time of treatment and the total germination in these reached 98 per cent for both fungi. The explanation of the results for the percentage of germination recorded in table 8 is that "Total" gives the figure for the total percentage of germinated spores in the hanging drop, "Growth" refers to the comparative lengths of the germ tubes, and "Killed" is the percentage of *germinated* spores which have been killed by the treatment. Since it has been shown that nongerminated spores are not all killed by treatment, a certain number of nongerminated spores in the suspensions here used must have germinated in the hanging drops after treatment, even though they had been rendered less resistant by their contact with the medium and the cell walls thereby having become softened. This complicated the observation of the number of spores killed, but, by using the mercuric chloride-treated spores as a check, a fair estimate could be obtained, as the germ tubes here had not elongated and collapsed. Where no increase in the length of the germ tubes took place and the amount of germination was the same as in the original suspensions complete killing occurred.

These treatments with germinated spores are duplicates of most of those recorded in tables 6 and 7. Certain similarities are noticed in the results obtained. Naturally mercuric chloride gave complete killing, and also sodium borate gave a greater relative amount of kill of *Penicillium digitatum* than of *P. italicum*. Like in the commercial treatments, the bicarbonates gave a greater relative amount of kill with the former than with the latter fungus. Further, an increase in concentration of treating substances and in time of treatment gave an increase in kill, both of the nongerminated and germinated spores in the mixed suspensions. However, no constant difference in the percentage of kill is noticed between the sodium

and potassium salts, while the same percentage of carbonate is considerably more effective than bicarbonate. This latter effect is undoubtedly due to the high hydroxyl-ion concentration of the carbonate solutions, for it has been shown by many workers that very few, if any, microorganisms can survive in the presence of a pH value above 10. Myers (14), working on the effect of alkaline washing solutions on *Bacterium coli* and *Bacillus cereus*, states that "powders that gave solutions of high pH values were distinctly more effective as germicides than those that gave solutions of low pH values." It is noticed that a 6 per cent solution of sodium bicarbonate, stronger than is used in practice, does not give a satisfactory kill even with the germ tubes exposed to it but that 10 per cent with 10 minutes treatment does.

That the toxicity of the sodium ion plays a very minor, if any, part in the action of sodium bicarbonate is shown by the fact that potassium bicarbonate is just as effective against germinated spores, and the potassium ion has been shown to have no inhibitory power at the highest concentrations used.

DISCUSSION

The whole question of hydrogen- and hydroxyl-ion studies of microorganisms is one which has brought forth many theories and opinions as to the reaction of fungi to these ions. There are, first, the general physiological reactions involved and, second, the specific variations in behavior of different fungi to these reactions. It seems agreed that in the case of acid solutions the inhibitory effect is mainly due to the action of the hydrogen-ion. Boeseken and Waterman (2), assuming that the protoplasm wall in microorganisms consists of an aqueous colloidal solution of lecithins, etc., mixed with albuminoid constituents, refer to toxic action of the hydrogen ion to the precipitation of these colloids. This precipitation may be regarded as a neutralization by the hydrogen ion of the negatively charged plasma colloids, as it were a physical process, and, because all highly dissociating acids act similarly, this explanation has been accepted. Whether the hydroxyl ion acts in the same manner, neutralizing the positively charged plasma colloids, has not yet been suggested, but it is highly probable that in this neutralization phenomenon we have the explanation of the action of the hydrogen and hydroxyl ions, provided they are present in sufficient concentration to overcome the resistance of the living protoplasm. Since the proteins in the protoplasm are amphoteric in nature, it may also be possible that the influence of the hydrogen and hydroxyl ions is such as to reverse the charge on certain proteins, causing a disruption of the protoplasmic system within the cell.

The differences shown by microorganisms in their tolerance to hydrogen and hydroxyl ions are characteristic of the individual species; sometimes

whole genera might react similarly and at other times closely related species might show marked differences. Zeller, Schmitz, and Duggar (29) state that "no general statement, in their opinion, could be made concerning the relation between hydrogen-ion concentration of the culture media and the growth of the wood-destroying fungi as a group." Such variation has been indicated to some extent between *Penicillium italicum* and *P. digitatum* in this work.

Whether germination or growth is used to measure the tolerance of fungi to deleterious substances the general trend of the results is the same, for when the spore wall breaks at the start of germination there is exposed a surface which is just as vulnerable as the tips of the mycelial strands. This would apply only to cases where the deleterious substance is present at the time of actual germination and not to such treatment experiments with nongerminated spores as reported here. The most toxic substances, such as mercuric chloride, kill the spore even before it swells, whereas with hydroxides and carbonates, when present in media of high pH value, a high percentage of the spores swell, even though final germination, as observed by the appearance of germ tubes, is inhibited.

The most striking point in the work with sodium bicarbonate is that its toxic action does not depend on the hydroxyl-ion concentration of its solution, as is the case with strong alkalies. This is shown by the fact that the spores of both fungi germinate in solutions of pH values of 9.0 to 9.6, while the pH of solutions of sodium bicarbonate is from 8.3 to 8.5. That the bicarbonates have an inhibitory power on the growth of fungi has been shown by several workers. It has been shown that enzymes secreted by Penicillium species are instrumental in bringing about the decayed condition of fruits. Nobécourt (15) found, however, that the addition of sodium bicarbonate to the active juice of fruits containing these enzymes makes it lose its property of destroying slices of sound tissue of these fruits. That in the case of Citrus the fruit itself has in no way been conditioned by treatment to resist the growth of the fungi within its tissues is shown by the fact that the blue and green molds do cause a small percentage of characteristic decay even after the fruit has been treated and 100 per cent decay if the rind tissue of the treated fruit is artificially inoculated. The fact remains that the bicarbonate ion in itself has the inhibitory power, and this must be ascribed either to its action on the enzymes or enzyme-secreting power of the protoplasm or to a direct toxic influence on the protoplasm itself. The latter view, in the opinion of the writer, is the more probable.

That the solutions of the hydroxides of sodium and potassium have a more toxic action than the carbonates on nongerminated spores of both Penicillium species was found to be the case upon experimentation. The

former solutions have a much higher pH value than the latter, and if the toxic action is ascribed to the hydroxyl-ion concentration it would be expected that the hydroxides would be more deleterious. The sodium salts, particularly the hydroxide, allowed a lower percentage of germination than the potassium salts, so that it seems that, while the hydroxyl ion is the main factor in inhibiting, the sodium ion assists therein, especially at the higher concentrations of salts.

Coming to the formulation of a theory as to how the actual process of decay prevention takes place the main factor to be borne in mind is that some unkilled spores of the decay-producing fungi are still present on the dry surface of the fruit following treatment. This seems reasonable because a 10 per cent solution of sodium bicarbonate in contact with spores for 10 minutes still allows 85 per cent of *Penicillium italicum* and 35 per cent of *P. digitatum* to germinate. It has been shown, however, that the same solution, almost a saturated one at ordinary temperatures, kills practically 100 per cent of the germinated spores. Further, no germination takes place in solutions of carbonates and bicarbonates plus sucrose, as against 40 per cent in the checks of sucrose in water. Water must be present for the germination of spores on the fruit, and, should water vapor condense thereon and the spores swell and start germinating, an almost saturated solution of bicarbonate will act on them, it being formed from the thin film of the salt left after drying. Death of most of the spores and germ tubes will result, and only those germ tubes can instigate decay which do not come into contact with this solution and which find a break in the rind to enter the albedo. That this percentage is small is shown by the small amount of decay found after mass treatment in packing houses.

It has been suggested that the rind of the fruit absorbs sodium bicarbonate during treatment and its presence therein inactivates the fungus should spores germinate. By making distilled-water extracts of the macerated rinds of well-washed lemons which had been soaked in 2, 3, and 4 per cent solutions of sodium bicarbonate for 24 hours it was found that the pH of the extracts was ± 5.5, the same as that of the untreated rinds. This pH value would not indicate the presence of free sodium bicarbonate in the rind of treated fruit.

The relation of temperature to the toxic action of the substances worked with here has not been included in these investigations. Weiss (25) showed that an increase in temperature lowered the hydrogen-ion concentration resistance of *Clostridium botulinum*, and the same would hold true for the hydroxyl-ion concentration. A temperature of 100° F. is used for the washing and treating solutions in packing houses, but this temperature *in itself* has no significant killing power on the spores. An increase in temperature increases the speed and probably the penetration power of the

ions, hence a greater toxicity is shown by toxic substances at higher temperatures. Thus it is to be expected that in the treating experiments recorded in this paper a higher percentage of kill would have been obtained by having the treating solutions at a higher temperature, but it is felt that the comparative kills would have remained almost the same. In view of the theory put forward as to the actual process of decay prevention, and, since immersion of fruit in water tends to make the rind slightly more leathery, it is further opined that the real value of warming the water in the treating tanks is that of bringing about a better adhesion of the sodium bicarbonate solution, thereby ensuring a more complete film after drying. This would apply also to treatment with sodium borate.

SUMMARY

In the commercial handling of citrus fruits they are treated before being packed with a 3 per cent solution of sodium bicarbonate for the prevention of the decay caused by *Penicillium italicum* (blue contact mold) and *P. digitatum* (green mold). Investigations as to just how this prevention is brought about, as well as experiments on the hydrogen-ion concentration relationships of the two fungi, are reported in this paper.

By growing the fungi on a Modified Duggar's Solution + orange extract in an apparatus which allowed the daily renewal of the medium under the mats and by weighing the dried mats after 8 days growth, a fairly wide optimum range of hydrogen-ion concentration tolerance for growth was observed, this being pH 2.9 to 6.5 for *Penicillium italicum* and pH 3.0 to 6.0 for *P. digitatum*. No mat at all was formed by the former fungus if the initial pH of the medium was below 2.1 and below 2.4 for the latter. The difficulty in determining the upper limit for mat formation lay in the submerged growth in this range, but the values ± pH 7.9 and ± pH 7.8, respectively, are offered for the two fungi as being near these limits.

Sodium citrate showed a marked inhibitory effect on the growth of *Penicillium italicum* by the sodium ion at a concentration of 20,000 p.p.m. in the medium and on *P. digitatum* at 10,000 p.p.m. The potassium ion has no such effect even at a concentration of 35,000 p.p.m. It is believed that the inhibitory effect on growth exerted by both the sodium and potassium chloride at a concentration of 10,000 p.p.m. of the cations is mainly due to the chlorine ion. The potassium ion does not inhibit germination at the highest concentrations used, but the sodium ion inhibits *P. italicum* markedly at 20,000 p.p.m. and *P. digitatum* at 15,000 p.p.m. Thus the sodium ion is more toxic to the latter fungus.

Certain correlations with the substances used and their toxic action on both germinated and nongerminated spores were observed. An increase in both the concentration of the treating substance and an increase in the

length of time of treatment gave a decrease in the percentage of germination of both fungi. No constant difference was noted in the toxic action of sodium and potassium salts, except perhaps with the treatment of nongerminated spores. The same concentration of carbonate was found to be considerably more toxic than a similar concentration of bicarbonate.

Comparing the effect on the spores of the two fungi sodium tetra-borate was relatively more toxic to *Penicillium digitatum* and the bicarbonates were relatively more toxic to *P. italicum*.

It is believed that the bicarbonate ion as such is toxic to the fungi, for its solution gives a pH value of $\pm$ 8.4, and that when the hydroxyl-ion concentration in a solution is large enough to give a pH value of 10+ the toxic property of such a solution lies in the hydroxyl ion, the destruction of the protoplasm being brought about by the neutralization of the positively charged colloids therein or by a reversal of the charge on amphoteric proteins in the protoplasm.

It is further postulated that the manner in which decay prevention is brought about is that when spores of the fungi on the rind of the fruit germinate or start to germinate a saturated solution of sodium bicarbonate formed from the thin film of salt left on the rind after treatment acts on the protoplast of the thin-wall germ tube or on the spore at the spot at which the wall is weakened for the emergence of the tube, death resulting.

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FURTHER STUDIES ON APHID TRANSMISSION OF PLANT VIRUSES[1]

ISMÉ A. HOGGAN[2]

A knowledge of the various modes of dissemination of plant-virus diseases is essential to the development of sound control measures. Insects, particularly aphids and leaf hoppers, are known to play a major rôle in the dissemination of many such diseases, and it appears to be quite generally assumed that tobacco mosaic is spread in this way. As has been pointed out in a recent paper (3), however, the peach aphid, *Myzus persicae* Sulz., one of two aphid species previously regarded as carriers of this disease, does not transmit the true tobacco-mosaic virus[3] between tobacco and other solanaceous plants, although it readily transmits the cucumber-mosaic virus between the same hosts. This, however, does not preclude the possibility of tobacco mosaic being disseminated by aphids of other species. In view of the practical importance of a thorough understanding of the insect relationships of this disease, it was considered desirable to investigate the relation of other species of aphids to its transmission.

It should be stated that tobacco, as grown in the field, does not appear to be a particularly favorable food plant for aphids in the United States. As far as can be ascertained from those familiar with this subject, these insects occur only relatively rarely on the tobacco crop in this country and are in no sense of the word pests on this plant. Nevertheless, it was believed important to determine the behavior of different aphids as potential agents in the dissemination of the tobacco-mosaic disease, and several species, that will feed and multiply more or less readily on tobacco in the greenhouse, have been secured from other hosts. The ability of these to transmit both the cucumber- and tobacco-mosaic viruses forms the subject of the present investigation, the results of which are recorded below.

APHID SPECIES INVESTIGATED

Of the three different strains of the peach aphid which were studied earlier in relation to virus transmission (3), although coming from widely separated localities, none was derived from tobacco while growing in the

[1] Cooperative investigations of the Wisconsin Agricultural Experiment Station and the Office of Tobacco and Plant Nutrition, Bureau of Plant Industry, United States Department of Agriculture.

[2] This investigation was undertaken on the suggestion of Dr. James Johnson, for whose valuable advice and criticism I wish to make due acknowledgement.

[3] *Tobacco virus 1*, as described by Johnson (4).

field. For purposes of comparison, therefore, further trials have been conducted with two other strains of this aphid derived from such a source, each strain being taken from a single tobacco plant growing in a separate field at Madison, Wisconsin. A third strain, obtained from cabbage in the greenhouse, has also been employed in certain trials.

Other aphid species studied in this investigation and the sources from which they were derived are as follows:

The pink and green potato aphid, *Macrosiphum solanifolii* Ashm.; collected on pigweed, *Amaranthus retroflexus*, on the University grounds, Madison, Wisconsin.

Myzus pseudosolani Theob.; taken from tomato in a greenhouse of the Department of Plant Pathology, University of Wisconsin.

Myzus circumflexus Buckt.; from a potato plant in a greenhouse of the Horticulture Department, University of Wisconsin.

The identifications of the species were kindly made or verified by Dr. A. A. Granovsky of the Department of Economic Entomology, University of Wisconsin.

METHODS

The methods employed in this work were similar to those of the preceding investigation on the transmission of viruses by the peach aphid (3) and need not be repeated in detail.

Stock colonies of each aphid species were maintained in separate insect-proof cages or chambers placed in different greenhouse units. The peach aphid was colonized on cabbage and the remaining three species were maintained on healthy, tuber-indexed potato plants. All transmission experiments were performed in a cool greenhouse in small, insect-proof cages, that were either fumigated or left empty for several days between use for successive trials. Special precautions were taken at all times to avoid any mixing of the aphid species. This greenhouse was maintained at a temperature of 65°–70° F., which proved very favorable for the aphids.

The ability of each species of aphid to transmit the cucumber- and tobacco-mosaic viruses was tested in comparative trials. The aphids were allowed to feed for two to five days on host plants infected with either virus and were then transferred to healthy plants by removing the leaves or stems on which they were feeding and placing them on a piece of paper laid on the new host. Large numbers of aphids in various stages of development were transferred thus to each plant, where they remained two or more days, and were subsequently destroyed by fumigation. In all trials, an equal number of control plants was treated in like manner with mosaic leaves or stems free from aphids. After fumigation, all plants were kept in a warm greenhouse (about 85° F.) in order to favor the rapid development of mosaic symptoms.

The cucumber- and tobacco-mosaic viruses used in the tests came from the same source as those employed in the previous investigation.

EXPERIMENTAL RESULTS

A limited number of trials was conducted with each of the two strains of peach aphid derived from tobacco while growing in the field, in order to determine the capacity of these strains for virus transmission. Several different host plants were included in the tests, the results of which are presented in table 1. These strains proved identical in their behavior with others previously studied. Each was found to transmit the cucumber-mosaic virus very readily between all hosts tested, 100 per cent infection occurring in most trials, while all control plants, with a single exception in the case of strain No. 2, remained healthy. On the other hand, no transmission of the tobacco-mosaic virus was obtained with either strain, whether from tobacco, Physalis, or *Nicotiana rustica*. In short, of the five different strains of the peach aphid which have been studied to date, all have been found to behave alike with respect to the transmission of the two viruses under consideration. The possible dissemination of tobacco mosaic by this species of aphid, at least between the different host plants which have been investigated, therefore appears remote.

Comparative trials have been conducted also of the transmission of the cucumber- and tobacco-mosaic viruses, from both tobacco and tomato, by *Myzus pseudosolani*, *Macrosiphum solanifolii*, and *Myzus circumflexus*. The results of these trials are summarized in table 2. It will be observed that each of these species resembled the peach aphid in readily transmitting the cucumber-mosaic virus between all hosts tested, *Myzus pseudosolani* and *Macrosiphum solanifolii* causing a particularly high percentage of infection in the majority of cases (Fig. 1). Moreover, there is no significant evidence in these results of any transmission of the tobacco-mosaic virus from tobacco by any species. The 5 tobacco-mosaic infections occurring in the transfers from tobacco, of which 3 were on the test plants and 2 were on the controls, are probably to be accounted for by accidental infection of one kind or another. The highly contagious nature of this mosaic disease should be borne in mind throughout, and, while the number of accidental infections occurring in the greenhouse has been greatly reduced through various precautionary measures in the growing and handling of the plants, it has not been found possible to eliminate such infections entirely in the ordinary routine of greenhouse work.

However, when the tomato plant was used as a source of the tobacco-mosaic virus a singular situation developed. Heavy infection with tobacco-mosaic invariably resulted when *Myzus pseudosolani* was transferred from diseased tomato to tobacco, as high a percentage of infection being obtained

TABLE 1.—*Comparative trials of the transmission of the cucumber- and tobacco-mosaic viruses by two strains of peach aphid (Myzus persicae) derived from tobacco in the field*[a]

Mosaic host (source of virus)	Host	Aphid strain No. 1				Aphid strain No. 2			
		Cucumber-mosaic virus		Tobacco-mosaic virus		Cucumber-mosaic virus		Tobacco-mosaic virus	
		Infested	Control	Infested	Control	Infested	Control	Infested	Control
Nicotiana tabacum	*N. tabacum*	30/29	30/0	30/0	30/0	30/29	30/1	30/0	30/0
Physalis pubescens	*N. tabacum*	5/5	5/0	5/0	5/0				
P. pubescens	*Capsicum annum*					5/5	5/0	5/0	5/0
N. rustica	*P. pubescens*					5/5	5/0	5/0	5/0
Total infections		35/34	35/0	35/0	35/0	40/39	40/1	40/0	40/0

[a] "Infested" denotes those plants to which aphids were transferred from a mosaic host; "control" denotes those plants which were treated with mosaic leaves free from aphids and which served as controls to the infested plants. In all fractions, the numerator represents the total number of plants either infested with aphids or treated as controls, as the case may be; the denominator the number of such plants which developed symptoms of mosaic.

TABLE 2.—*Comparative trials of the transmission of the cucumber- and tobacco-mosaic viruses by different aphid species*[a]

TABLE 2.—*Comparative trials of the transmission of the cucumber- and tobacco-mosaic viruses by different aphid species*[a]

Aphid species	Mosaic host (source of virus)	Mosaic virus	Hosts: *N. tabacum* Infested	*N. tabacum* Control	*L. esculentum* Infested	*L. esculentum* Control	*P. pubescens* Infested	*P. pubescens* Control	*C. annuum* Infested	*C. annuum* Control	Total infections: Infested	Total infections: Control
Myzus pseudosolani	*Nicotiana tabacum*	Cucumber	50 / 46	50 / 1	20 / 11	20 / 0					70 / 57	70 / 1
		Tobacco	50 / 2	50 / 2	20 / 0	20 / 0					70 / 2	70 / 2
	Lycopersicon esculentum	Cucumber	20 / 16	20 / 1							20 / 16	20 / 1
		Tobacco	20 / 17	20 / 0							20 / 17	20 / 0
Macrosiphum solanifolii	*N. tabacum*	Cucumber	50 / 44	50 / 0	10 / 7	10 / 0	10 / 7	10 / 1	5 / 3	5 / 0	75 / 61	75 / 1
		Tobacco	50 / 1	50 / 0	10 / 0	10 / 0	10 / 0	10 / 0	5 / 0	5 / 0	75 / 1	75 / 0
	L. esculentum	Cucumber	30 / 28	30 / 0							30 / 28	30 / 0
		Tobacco	65 / 31	65 / 0							65 / 31	65 / 0
Myzus circumflexus	*N. tabacum*	Cucumber	50 / 38	50 / 0							50 / 38	50 / 0
		Tobacco	50 / 0	50 / 0							50 / 0	50 / 0
	L. esculentum	Cucumber	30 / 22	30 / 0							30 / 22	30 / 0
		Tobacco	30 / 13	30 / 0							30 / 13	30 / 0
Myzus persicae	*L. esculentum*	Cucumber	30 / 30	30 / 0	10 / 8	10 / 0					40 / 38	40 / 0
		Tobacco	65 / 2	65 / 0	10 / 0	10 / 0					75 / 2	75 / 0

[a] See footnote to table 1.

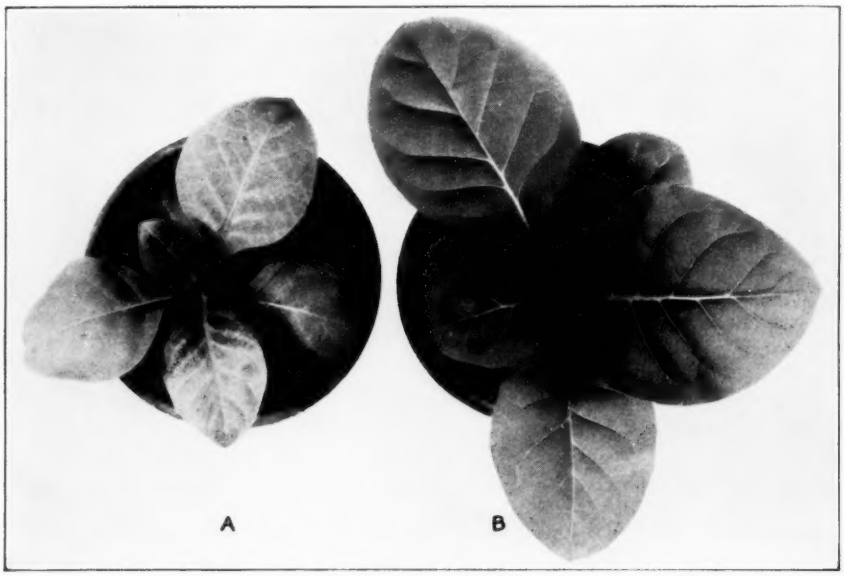

FIG. 1. Tobacco plants showing approximate size and type used in the transmission experiments. A, cucumber mosaic on tobacco, transmitted by *Macrosiphum solanifolii*. B, healthy control plant.

as in the corresponding trials with the cucumber-mosaic virus (Table 2). Since all control plants remained healthy, this infection could not be ascribed in any way to the portions of tomato stems or leaves on which the aphids were transferred, for the control plants were similarly treated. Repeated trials consistently yielded abundant infection in all transfers of this aphid from tomato, leading to the conclusion that in this case the aphid was able to act as a vector of the tobacco-mosaic virus. Subsequent trials with *Macrosiphum solanifolii* and *Myzus circumflexus* showed that these species, too, were able to transmit the tobacco-mosaic virus from tomato, although not from tobacco. With these latter species, however, a distinctly lower percentage of infection was obtained (between 40 and 50 per cent).

In the previous investigation of the peach aphid (3), although a number of different host plants were tested, tomato was not included among these, since it was not considered a satisfactory food plant for the species. The results recorded above, however, rendered necessary a determination of the behavior of this aphid also with regard to the transmission of viruses from tomato, and, by using very young tomato plants and large numbers of aphids, fairly adequate tests could be made (Table 2). The strain of peach aphid employed in these tests was derived from cabbage in the greenhouse. Here, however, repeated trials with the tobacco-mosaic virus gave only two

infections on tobacco in a total of 65 plants, although in corresponding trials with the cucumber-mosaic virus 100 per cent infection was obtained. Whether or not these two tobacco-mosaic infections were due to aphid transmission appears very doubtful, in spite of the fact that all control plants remained healthy. In any case, the peach aphid did not transmit the tobacco-mosaic virus from tomato to any appreciable extent, differing in this respect, at least in degree, from the other species under investigation.

In order to confirm these somewhat peculiar results, more strictly parallel tests were performed with *Myzus pseudosolani*, comparing tobacco and tomato as respective sources of the tobacco-mosaic virus. In these trials, young tobacco and tomato plants were inoculated with virus from a single sample of mosaic-plant extract and, as soon as typical symptoms had developed, were infested simultaneously with large numbers of aphids from the same stock colony. These two host species were isolated in separate insect cages. After several days, the aphids were transferred simultaneously and in great quantities to healthy plants of different species, such as tobacco, *Nicotiana rustica*, etc.; the insects were subsequently destroyed by fumigation and the plants observed for the appearance of disease symptoms. In brief, conditions were exactly duplicated in the two trials, the only difference being in the species of host plant employed as a source of the virus. The results of these trials are presented in table 3. Transfers of aphids from tomato yielded abundant tobacco-mosaic infection on all hosts tested; whereas corresponding transfers from tobacco gave almost no infection. From tomato, of a total of 74 plants tested, 59 showed mosaic infection, with a single infection in the controls; while from tobacco, of a total of 86 plants tested, only 4 became infected, and 1 plant also in the controls. Here, again, it is questionable whether or not the 3 infections occurring on the infested plants only, in the transfers from tobacco, are the result of aphid transmission. The writer is inclined to believe that they are due to accidental infection from other sources, although it is possible that the aphid may have been able, on extremely rare occasions, to transmit the virus from this host. The contrast between the two host species as sources of the tobacco-mosaic virus in relation to the amount of virus transmission is none the less striking. It should be stated that *M. pseudosolani* thrives equally well on both tobacco and tomato; hence these results cannot be accounted for on the basis of suitability of the species as a food plant for the aphid.

The variety of tomato employed in the preceding experiments was Hudson Valley Maid, a potato-leaf variety. Five other varieties have also been tested with respect to tobacco-mosaic transmission by *Myzus pseudosolani*, namely, Marglobe, Bonny Best, Red Pear, Red Cherry, and Vaughan's Model. All these have yielded high percentages of infection, no

TABLE 3.—*Comparative trials of the transmission of the tobacco-mosaic virus by Myzus pseudosolani from tobacco and from tomato*[a]

Mosaic host (source of virus)	Hosts										Total infections	
	N. tabacum		*L. esculentum* (Hudson Valley Maid)		*L. esculentum* (Marglobe)		*N. rustica*		*P. pubescens*			
	Infested	Control	Infested	Control	Infested	Control	Infested	Control	Infested	Control	Infested	Control
Nicotiana tabacum	36/2	36/0	20/0	20/0	10/1	10/0	10/1	10/1	10/0	10/0	86/4	86/1
Lycopersicon esculentum	24/22	24/1	20/13	20/0	10/8	10/0	10/10	10/0	10/6	10/0	74/59	74/1

[a] See footnote to table 1.

significant differences in the amount of resultant infection being noted between any of the varieties (Table 4). In all, of a total of 130 transfers

TABLE 4.—*Comparative trials of the transmission of the tobacco-mosaic virus by Myzus pseudosolani from different host species*[a]

Mosaic host (source of virus)	Host *N. tabacum*	
	Infested	Control
Nicotiana tabacum	36/2	36/0
Lycopersicon esculentum (Hudson Valley Maid)	24/22	24/1
" (Marglobe)	36/33	36/0
" (Bonny Best)	20/17	20/0
" (Red Pear)	10/10	10/0
" (Vaughan's Model)	10/10	10/0
" (Red Cherry)	10/8	10/0
Nicotiana rustica	30/0	30/0
Solanum nigrum	60/7	60/0
Solanum melongena	30/0	30/0
Solanum tuberosum (Green Mountain)	30/0	30/0
Martynia louisiana	30/2	30/0

[a] See footnote to table 1.

of this aphid from tomato to tobacco, 117 plants became infected with the tobacco-mosaic virus, as compared with a single plant in 130 in the control series. Corresponding figures for the transfers from tobacco to tobacco show only 4 plants infected out of 86, with 2 out of 86 in the controls.

As a consequence of these results, it became desirable to investigate the relation of other solanaceous plant species to the transmission of the tobacco-mosaic virus by *Myzus pseudosolani*, in order to determine whether or not the tomato was exceptional in this respect. The following species have been tested: *Nicotiana rustica;* nightshade, *Solanum nigrum;* eggplant, *S. melongena;* potato, Green Mountain variety; also *Martynia louisiana*[4] of the family Martyniaceae. Other hosts, such as Physalis, pepper, etc., were not included since they were found to be unsatisfactory food plants for this aphid. In these trials, little or no evidence was obtained of any tobacco-mosaic transmission by *Myzus pseudosolani* (Table 4). In the transfers from *S. nigrum*, however, 7 plants out of 60 became infected, suggesting an occasional transmission of the virus from this host. Two infections in 30 plants will be noted also in the transfers from *Martynia louisiana*, while from *N. rustica*, potato, and eggplant there was no indication of any virus transmission. Tomato is, therefore, the only host now known from which *Myzus pseudosolani* will transmit the tobacco-mosaic virus with any readiness, and it would appear that this aphid is to be regarded as a carrier of the virus only in so far as certain specific host plants are concerned.

A similar situation has been found with respect to the transmission by *Myzus pseudosolani* of a form of "yellow tobacco mosaic," obtained from a tobacco plant growing in the field at Madison, Wisconsin. This mosaic is apparently identical with that described by Johnson (4) and others, the virus closely resembling in its properties that of ordinary tobacco mosaic. This virus also was found readily transmissible by *M. pseudosolani* from tomato, but not at all from tobacco. In transfers from tomato to tobacco, of 50 plants tested, 35 became infected with yellow tobacco mosaic, while all control plants remained healthy. In corresponding transfers from tobacco, of 50 plants tested, none became infected nor were there any infections on the controls. These studies, therefore, suggest that when determining the ability of certain insects to transmit particular viruses, the species of host plant serving as source of the virus may need to be taken into consideration.

Transmission of the yellow tobacco-mosaic virus by *Myzus pseudosolani* is illustrated in figure 2. This aphid is somewhat peculiar in that it is markedly injurious to the tissues of the host plant, quite apart from any mosaic transmission, presumably due to the presence of some deleterious substance or substances in the saliva. This effect is first seen as a discol-

[4] The writer was informed by Dr. Helen A. Purdy in 1927 that tobacco mosaic was transmissible to this species. This fact has been confirmed and the presence of characteristic cell inclusions (x-bodies and striate material) has been demonstrated in the host tissues.

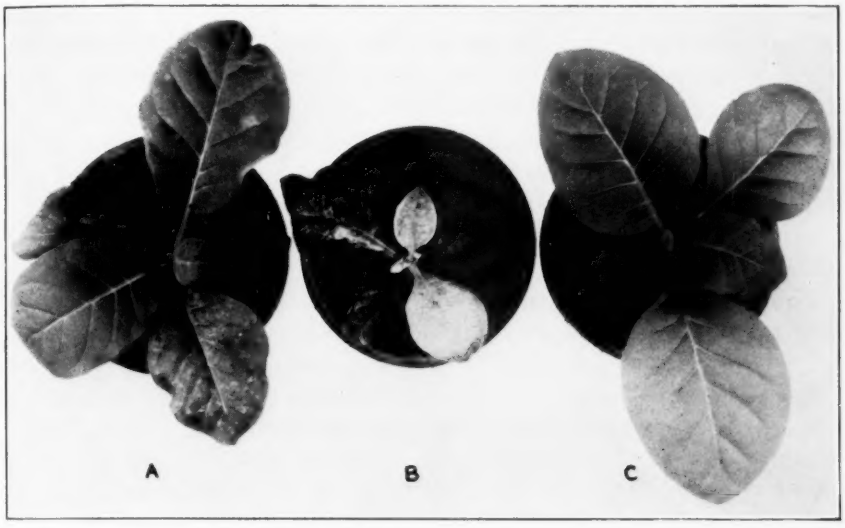

FIG. 2. A, tobacco plant to which *Myzus pseudosolani* was transferred from yellow tobacco-mosaic tobacco, showing aphid injury on lower leaves but no mosaic infection. B, yellow tobacco mosaic on tobacco, transmitted by *M. pseudosolani* from infected tomato. Note aphid injury on lower leaves. C, healthy tobacco plant, control to B.

oration of the tissues in the form of small, pale yellowish spots around the point of entrance of the insect's proboscis; circular on the leaf lamina, and in the form of narrow bands running along the vein, when this is punctured. As the insects continue to feed, the spots enlarge and become golden brown. If the plant is heavily infested, they may coalesce to form large, necrotic areas, frequently resulting in the death of the entire leaf. Eventually the whole plant may be killed. After the aphids have been destroyed by fumigation, the spots may continue to enlarge for a time and the color to intensify, but, if the plant survives, the new leaves develop normally without any spotting, and the plant may eventually completely recover. This injury has been observed on all host plants on which the aphid has fed. It is illustrated on tobacco in figure 2, A. Plant B shows a similar injury, followed by infection with yellow tobacco mosaic, while C is a normal, healthy plant.

DISCUSSION

The development of adequate control measures for the mosaic disease of tobacco is dependent upon a variety of factors. In this connection the particular virus involved naturally requires consideration; however, it appears that, although tobacco is susceptible to a number of different viruses, the ordinary tobacco-mosaic virus (*tobacco virus 1*) (4) is almost universally the cause of the disease so far as field conditions are concerned.

Aphids have been regarded as of major importance in the dissemination of this disease, and *Myzus persicae* and *Macrosiphum tabaci* Pergande have been reported as active carriers of the virus (1, 2). Assuming *Macrosiphum tabaci* to be synonymous with *M. solanifolii,* as is stated by Patch (6, p. 29), it has now been shown that neither of these two species as used in these experiments will transmit the ordinary tobacco-mosaic virus from tobacco, and it seems likely that, where transmission of a mosaic disease of tobacco appeared to be due to these aphids, some other virus affecting this host was actually involved.

Two other aphid species in addition to those named above also have failed to transmit the true tobacco-mosaic virus from tobacco. Furthermore, as has already been pointed out, aphids are not of common occurrence on tobacco in this country. Wilson and Vickery (7) list two species only as reported to occur on this host, namely: *Macrosiphum tabaci* and *Rhopalosiphum (Myzus) persicae* Sulzer; and, if other species attack the tobacco plant, they do not appear to be abundant. When all these facts are taken into consideration, therefore, the probability of tobacco mosaic being disseminated to any extent by aphids appears remote so far as transmission from tobacco is concerned. Moreover, the occurrence and spread of tobacco mosaic in the field can be adequately accounted for in other ways (5), due in large measure to the highly infectious nature of the virus, together with its remarkable resistance to unfavorable conditions.

On the other hand, since it has been shown that certain aphid species are able to transmit the tobacco-mosaic virus from tomato, it is possible that these insects may be responsible for some dissemination of the disease on tobacco where the two crops are grown in close proximity, and it is not unlikely that they may play an important part in disseminating the disease on the tomato crop, either in the greenhouse or in the field.

Turning to the more fundamental considerations arising from these investigations, the remarkable influence of the species of mosaic host in determining the amount of tobacco-mosaic transmission brought about by certain aphids is deemed worthy of attention. Whether this is an entirely exceptional situation or not is, of course, an open question at the present time. The fact that *Myzus pseudosolani* will so readily transmit the tobacco-mosaic virus from tomato would seem to indicate that there is nothing inherent in the aphid itself to account for its failure to transmit the same virus from tobacco. It would appear, rather, that for some reason the virus is not available to the aphid in the tobacco plant; in other words, that it may be a question of the distribution of the virus within the host. If this is so, then there is no evidence in these studies which would indicate a biological rather than a mechanical relationship between the virus and the aphid vector. Although aphids are believed in general to feed upon

the phloem tissues of the host plant, this may not always be the case, and it is conceivable that these phenomena may be bound up with the nature of the tissues tapped by the aphid in the respective host plants. Further investigations along these lines appear to be necessary before any definite conclusions may be reached.

SUMMARY

1. An investigation has been made of several different species of aphids, in order to determine their ability to transmit the virus of ordinary tobacco mosaic.

2. Two strains of peach aphid, *Myzus persicae*, derived from tobacco, growing in the field, failed to transmit the tobacco-mosaic virus between tobacco and other solanaceous host plants, although they readily transmitted the cucumber-mosaic virus between the same hosts.

3. Three other aphid species, namely, *Myzus pseudosolani*, *Macrosiphum solanifolii*, and *Myzus circumflexus*, were shown to transmit the cucumber-mosaic virus readily from tobacco and from tomato.

4. The above three species appear unable to transmit the tobacco-mosaic virus from tobacco. They will, however, transmit this virus from tomato, *Myzus pseudosolani* causing very high percentages of infection. Evidence has been obtained to indicate that the peach aphid, on the other hand, does not transmit this virus from tomato, or on only very rare occasions.

5. Transmission of the tobacco-mosaic virus by *Myzus pseudosolani* was demonstrated from six different varieties of tomato, while from four other host species in addition to tobacco no transmission was obtained. From *Solanum nigrum* there was evidence of occasional transmission of the virus.

6. *Myzus pseudosolani* was shown to transmit also a form of "yellow tobacco mosaic" from tomato, but not from tobacco.

7. The evidence thus far obtained indicates that aphids are unlikely to be responsible for any dissemination of ordinary tobacco mosaic, so far as transmission from tobacco is concerned. They may, however, play an important part in the dissemination of this disease on tomatoes, or from tomato to tobacco where these two crops are grown in close proximity.

8. The failure of *Myzus pseudosolani* to transmit the tobacco-mosaic virus from tobacco may be explained on the assumption that the aphid does not extract the virus from those tissues of the tobacco plant on which it feeds.

UNIVERSITY OF WISCONSIN,
MADISON, WISCONSIN.

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LIGHTNING INJURY OF POTATOES

GEORGE F. WEBER

During the past several growing seasons the writer's attention has been called to a specific type of injury of growing potato plants. The injury has appeared in Florida fields where the plants were about two thirds mature, usually during the month of March. After careful consideration of the phenomenon from the viewpoint of a disease caused by a parasitic organism, environmental conditions, physiological disturbances or mechanical injury, together with information supplied by the growers, it was concluded that lightning was the cause of the trouble.

A detailed description of a single occurrence is as follows: During the latter part of March, 1929, an affected area appeared in a 6-acre field of Spaulding Rose potatoes about 70 days old owned by a Mr. Cox near La Crosse, Florida. The field was surrounded on three sides by pine woods and on the fourth side by another field. The affected area was almost circular, measuring 70 by 80 feet in diameter, with the greater diameter parallel with the rows. The soil in the field belonged to the Portsmouth series and was a rather moist, heavy, black sandy loam. The potato plants were in a vigorous condition and were about 16 to 20 inches high with an occasional blossom showing. The injury to the potato plants was not noticed by Mr. Cox until 4 days after the passing of a furious local thunderstorm, which he believes marked the time of the bolt, since showers are rare during the spring and none had preceded this one over a period of 3 weeks. None of the plants were entirely killed and only the plants in a small area, approximately 10 feet in diameter, were prostrate when first observed by the writer 4 days after the thunderstorm (Fig. 1). Outside of this area, none of the plants became prostrate before they were harvested. This condition differs from the case of lightning injury reported by Jones and Gilbert,[1] as they stated that the potato plants were killed in a spot about 12 feet in diameter and that they observed other spots that varied from 8 to 10 feet to 2 to 4 rods in diameter.

Outside of this small area the extent of injury appeared to decrease in direct proportion to the distance from the center, that is, a smaller portion of the stems of the plants had collapsed. For instance, at the periphery of the area, only an occasional growing tip showed any injury, while as one approached the center of the spot more and more of the tops of the plants were injured from the top downward and were shriveled and drooped.

[1] Jones, L. R., and W. W. Gilbert. Lightning injury to potato and cotton plants. Phytopath. 5: 94–102. 1915.

FIG. 1. A. Effect of lightning on potato vines, showing sections of rows of affected potato plants. B. Affected plants in detail, showing collapsed condition with unaffected petioles and leaves

About half way from center to edge of the spot the plants were affected over half their height from the top down. This is the reverse of the condition reported by Jones and Gilbert,[2] as they stated that "the injured plants still living showed partial collapse and death of the stem from a little below the ground line upward through one half their length, more or less. The tips were living and below ground, the base of the stems, roots, stolons and young tubers seem uninjured." Orton[3] also stated that the stems of the more severely affected plants were collapsed from near the ground upward for several inches and often nearly to the tip.

In the case herein reported no effect of the lightning bolt on the soil in the way of physical disturbances was discovered. A number of hills of potatoes were dug in different parts of the affected area and no marks or signs were discovered on the roots or tubers that could possibly be attributed to lightning.

Furthermore, the stems of the prostrate plants appeared normal below the soil surface; above this point they were shriveled and prominently ribbed and more or less flat (Fig. 2). This collapsed condition included the whole stem from the soil line to the growing tip. The main branches which originated from the main stalk above the soil line were usually not affected but the ones which originated below the soil line were injured. The leaf petioles had also collapsed in some instances, but generally they were apparently unaffected, as they were turgid, brittle, and without unnatural symptoms. Likewise, the leaf blades appeared to be uninjured, even the ones on prostrate stems. The affected plants were green and vigorous, even though they were flattened out and prostrate on the soil surface. In contrast to this, Jones and Gilbert[4] stated that potato plants struck by lightning wilted and died within 24 hours. They also stated that the shriveled stems turned brown progressively above and below the point of injury.

The most peculiar and striking condition in relation to the above situation was that, even though the plants in the center of the spot were so injured as to be prostrate and their stems shriveled and flattened, they still retained their foliage in a healthy, turgid condition indistinguishable from potato leaves outside the injured area.

In order to make a comparison of injured and uninjured stems, specimens were collected within and outside of the affected area. Cross sections were made of the stems of the plants approximately the same distance from the growing tip on injured and uninjured plants that compared favorably

[2] Jones, L. R., and W. W. Gilbert. Lightning injury to herbaceous plants. Phytopath. 8: 270–282. 1918.

[3] Orton, C. R. Lightning injury to potato and cabbage. Phytopath. 11: 96–98. 1921.

[4] *Loc. cit.* 1915.

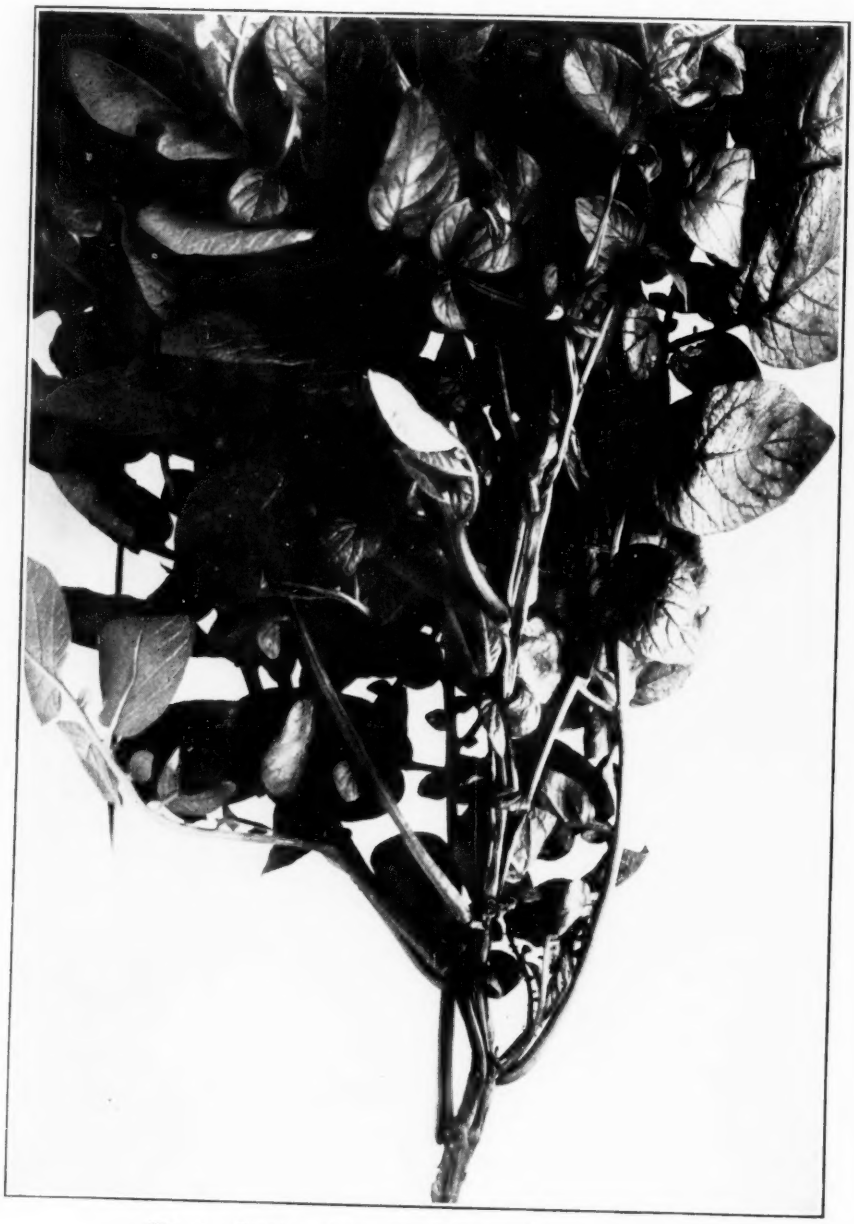


FIG. 2. Single potato stem, showing injury in detail.

with each other in height, diameter of underground stem, and amount of foliage. These cross sections are shown in figure 3. The corresponding parts are designated as (a) epidermis, (b) collenchyma, (c) parenchyma of cortex, (d) pericycle, (e) cambium, (f) vascular bundle, and (g) pith.

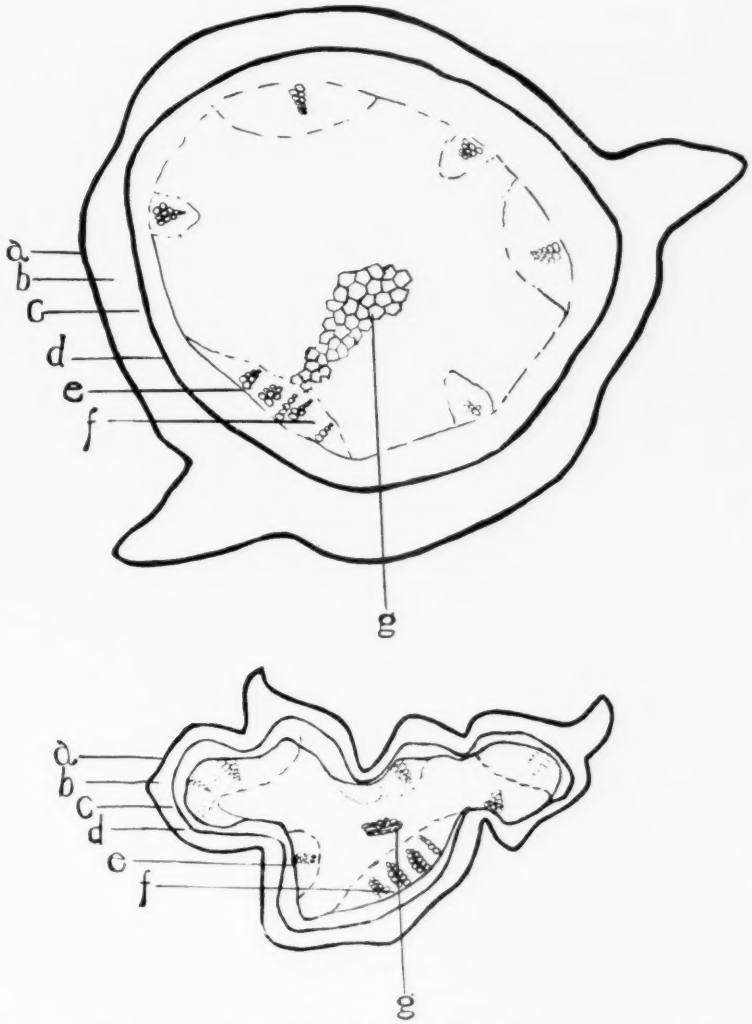


FIG. 3. Cross sections of uninjured (above) and lightning-injured (below) potato stems, showing corresponding morphological characters of each.

a. epidermis
b. collenchyma
c. parenchyma of cortex
d. pericycle
e. cambium
f. vascular bundle
g. pith

The greatest contrast appears in the pith, which has almost totally collapsed in the injured plant. No sign of hollow stem was discovered, as described by Jones and Gilbert[5] in potatoes and by Brown and Gardner[6] in tomatoes. On the other hand, the entire stems of these potato plants collapsed, becoming more or less irregularly flattened. The collenchyma and parenchyma of the cortex also showed a decided collapse, while the vascular bundles occupied very nearly the same amount of area in the injured stems as in the healthy ones, showing that this tissue suffered very little injury. This may account for the turgidness of the foliage of the prostrate plants.

The total collapse of the pith caused the stem to become more or less flattened. The internal portion consisted of a closely compressed tough layer of collapsed pith cells. Upon soaking in water, the cut section, as shown in the figure, tended to swell and partially regained its normal size. It did not fully recover, however, after 24 hours.

FLORIDA AGRICULTURAL EXPERIMENT STATION,
GAINESVILLE, FLA.

[5] *Loc. cit.* 1915.

[6] Brown, H. D., and Max W. Gardner. Lightning injury to tomatoes. Phytopath. 13: 147. 1923.

PATHOGENICITY OF BACILLUS AMYLOVORUS ON SPECIES OF JUGLANS[1]

CLAYTON O. SMITH

The pear blight organism, *Bacillus amylovorous* (Burrill), a well-known parasite, attacks several species of the Rosaceae. A list of susceptible plants has been prepared by Rosen and Groves (2), but the list includes no hosts outside of the Rosaceae. Burrill (1, 2) and a number of other early investigators reported the occurrence of pear blight on a number of plants, among which were *Juglans cinerea* L., butternut; *Juglans* sp., walnut; and *Hicora* sp., hickory. These early observations were without convincing evidence as to the nature of the maladies. An excellent summary of them has been presented by Snow (4). While no experimental proof has been published of *B. amylovorus* attacking any host except within the family Rosaceae, its successful inoculation[2] on Juglans, as later reported in this paper, would suggest that susceptible plants may be found in other of the botanical families.

The cultures used in this study were isolated from fire-blight lesions of pear trees and a species of Cotoneaster. These cultures, when inoculated

TABLE 1.—*Inoculations on Jugulans with the pear-blight organism, Bacillus amylovorus*

Species	Tissue	Inoculations		Results	
		Manner	Number	Positive	Negative
J. regia	Nuts	Puncture	20	20	0
	Nuts	Contact	10	6	4
	Shoot	Puncture	20	16	4
J. nigra	Nuts	"	20	15	5
	Shoot	"	10	4	6
J. californica	Nuts	"	5	3	2
J. Hindsii	Nuts	"	10	10	0
	Shoot	"	40	34	6
J. Sieboldiana	Nuts	"	20	20	0
	Shoot	"	3	1	2
J. cordiformis	Nuts	"	20	15	5
	Shoot	"	3	0	3

[1] Paper No. 230, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station.

[2] The pathogenicity of *Bacillus amylovorus* on Jugulans was first demonstrated, accompanied by inoculations, by M. C. Goldsworthy (data not published), and was called to the writer's attention about 1926.

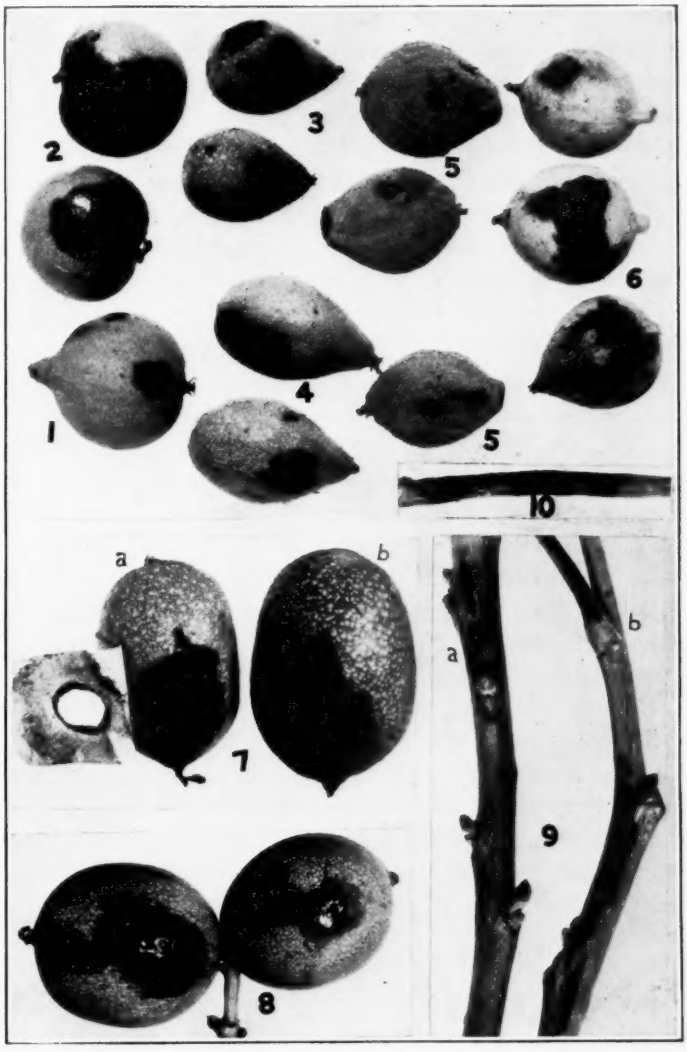
into pear shoots, reproduced the disease in its typical form. The cultures, so far as tested, appeared to be typical of the pear-blight organism.

The inoculation tests were made in 1927 to 1930 on the succulent twigs and, as far as possible, on the nuts of the following species: *Juglans californica* Walt., *J. Hindsii* Sarg., *J. insularis* Grisebach (a Cuban species), *J. major* Heller, *J. nigra* L., *J. regia* L., *J. Sieboldiana* Maxim., *J. Sieboldiana* var. *cordiformis* Makino, *J. mandschurica* Maxim., *J. pyriformis* Liebm. (from Mexico), and some hybrids between some of these species. A partial summary of these inoculations is given in table 1.

INOCULATIONS ON JUGLANS REGIA

The inoculations on the nuts were made by puncture in June and July. Three punctures were made on each of five nearly full-size nuts. In three days from inoculation definite lesions 5 mm. in diameter had formed about the punctures, and in five days the infected areas were 40 to 50 mm. in diameter (Fig. 8). The lesions in most cases had coalesced. The infected necrotic area was darker than normal tissue and eventually had a somewhat water-soaked appearance. The oldest, central part of the lesion was nearly black, while between this and the normal tissue there was a diseased zone of a somewhat deeper green than that of the normal tissue. Some of the infected nuts, when placed in a moist chamber over night, developed sticky drops on the surface, a well-known characteristic of this organism. The tissue of the infected nuts soon showed a shrivelled appearance. With a culture of *Bacillus amylovorus* isolated from *Cotoneaster* sp., the tests were duplicated on Eureka walnuts in 1929, and were repeated in 1930 with the same definite lesions. In these inoculations under field conditions no protection was given the infection courts on the nuts.

The fruits of the English walnut, when inoculated, are apparently as susceptible to *Bacillus amylovorus* as they are to *Bacterium juglandis* (Pierce), a proven pathogene of *J. regia*, and generally ascribed as the sole cause of the destructive disease known as walnut blight. Inoculations by brushing some of the bacterial growth of *B. amylovorus* on the surface of the nuts with a camel's-hair brush, without covering, gave negative results. When the nuts were thus brushed and protected with adhesive tape to which was fastened a circle of filter paper moistened with the bacterial growth, the results in six cases were positive (Fig. 7, also Table 1). In some of the other contact inoculations the results were slight, with some blackening of the surface, or altogether negative. The results are not entirely conclusive that the organism can always enter through the stomata of the nut, but these inoculations would indicate that this sometimes takes place.



FIGS. 1–6, inoculations with *Bacillus amylovorus* on nuts of Juglans. 1, *J. major;* 2, two nuts of paradox hybrid (*J. Hindsii* × *J. regia*); 3, two nuts of *J. Sieboldiana* var. *cordiformis;* 4, two nuts of *J. Seiboldiana;* 5, three nuts of *J. nigra;* 6, three nuts of *J. Hindsii.* 7–8, inoculation on *Juglans regia.* 7a, inoculation by placing bacterial growth on surface and on a circle of filter-paper fastened to adhesive tape and the whole fastened to nut; 7b, nut inoculated like nut at left, only punctured; 8, two nuts inoculated by puncture. 9, shoots of *Juglans regia* inoculated by puncture. Shoot at right shows inoculated petiole and large lesions on succulent growth; shoot at left part of that at right. Note smaller lesions. 10, puncture inoculation on twigs of *Juglans Hindsii.* Photographed after 14 days.

Puncture inoculations on the succulent twigs gave definite lesions (Fig. 9) 15 to 22 mm. long and of a blackish color. On the more woody tissue the lesions were much smaller, as indicated in figure 9, a. These lesions on the English walnut appear to be similar to those reported (5, p. 353) as being produced on this host by *Bacterium juglandis*. While no experimental evidence has been found to show that *Juglans regia* is attacked in nature by *Bacillus amylovorus*, yet such relations should be looked for in future isolations from walnut-blight tissue. Inoculations on succulent pear shoots with *Bact. juglandis* have all been negative.

INOCULATIONS ON OTHER SPECIES OF JUGLANS

The nuts of *Juglans californica*, *J. Hindsii*, *J. major*, *J. nigra*, *J. Sieboldiana*, *J. Sieboldiana* var. *cordiformis* and some paradox hybrids (*J. Hindsii* × *J. regia*), also *J. californica* × *J. regia* and Royal hybrid (*J. nigra* × *J. regia*) were inoculated by puncture in the open field with *Bacillus amylovorus*. In 5 to 10 days definite black lesions, 10 to 20 mm. or more in diameter, had developed on all the nuts of the above species, except on *J. nigra*, where the lesions were smaller, about 5 mm. in diameter. (See Figs. 1–6.) This small size is believed to be due to the more mature hard tissue of the larger nuts.

The shoots (Fig. 10) of all these species can be readily infected artificially on the more succulent parts, while the more woody tissue is slightly infected. Probably the slight darkening of some of these latter inoculations extends to little more than the injured tissue. In addition to the above susceptible species, shoots of other species, *Juglans insularis*, *J. pyriformis*, and *J. mandschurica*, were inoculated with apparently negative results. Inoculations on shoots of *Hicora pecan* Brit. with *Bacillus amylovorus* were negative.

REISOLATIONS AND REINOCULATIONS

Cultures were made from selected artificial inoculations on the nuts and shoots of *Juglans regia* and from them *Bacillus amylovorus* was reisolated. Reinoculations into branches of *Pyrus* sp. gave positive results. The recovery of the organism from the inoculation on other species of Juglans was not attempted.

SUMMARY

Bacillus amylovorus, a well-established pathogene of different species of the Rosaceae, when inoculated into tissues of Juglans, has produced blackish necrotic lesions 5 to 20 mm. in diameter. Under natural conditions the organism has never been demonstrated as attacking any species of Juglans.

The nuts of the seven species, including *Juglans regia* and three types of hybrids between these species, were successfully inoculated, forming lesions not distinguishable from those formed by *Bacterium juglandis*, a pathogene of *J. regia*. The succulent shoots of *J. regia* and most of the other species, when inoculated, proved to be susceptible, forming lesions 10 to 20 mm. in diameter. The epidermis of the shoot was the part attacked and darkened. In the nut the shell seemed to be the part involved.

GRADUATE SCHOOL OF TROPICAL AGRICULTURE
AND CITRUS EXPERIMENT STATION,
UNIVERSITY OF CALIFORNIA,
RIVERSIDE, CALIFORNIA.

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TRANSMISSION OF TOBACCO RING SPOT BY SEED OF PETUNIA[1]

R. G. Henderson

Reports of seed-borne virus diseases of leguminous plants are not uncommon. In fact, it seems that the virus diseases of legumes are transmitted through the seed in the majority of cases. For instance, McClintock (7), in 1917, reported that lima-bean mosaic was seed-borne. Two years later Reddick and Stewart (10) discovered that bean mosaic was transmitted through the seed, and their results have since been confirmed by the work of Archibald (1), Pierce and Hungerford (9), Fajardo (4), and others. Seed transmission of soy-bean mosaic was reported by Gardner and Kendrick (5) in 1921 and by Kendrick and Gardner (6) in 1924. Dickson (2) increased this list by reporting seed transmission of mosaic for pea, red clover, alsike clover, and sweet pea in 1924.

The brief statement given above will suffice to show that seed transmission seems to be the rule rather than the exception for virus diseases of leguminous plants. This rule, however, will not hold good for the virus diseases of other than leguminous plants. A casual review of the literature reveals the fact that virus diseases of non-leguminous species are very seldom transmitted through the seed, and, in the few cases reported, the amount of infection thus transmitted has been very low in comparison with that reported for the virus diseases of legumes. Doolittle and Gilbert (3), in 1919, for example, reported that out of a total of 130 plants of wild cucumber, *Micrampelis lobata*, grown from seed collected from mosaic-infected plants, only ten developed mosaic. Newhall (8), in 1923, reported seed transmission of lettuce mosaic to the extent of about 3 per cent.

In a recent issue of The Plant Disease Reporter, Valleau (11) reported that evidence had been obtained to show that tobacco ring spot is transmitted through tobacco seed. The results of the writer's studies on tobacco ring spot are not in agreement with Valleau's statement. The writer, however, has found that tobacco ring spot is very definitely transmitted through the seed of garden petunia, *Petunia violacea* Lindl., and the purpose of this paper is to present his data on this subject.

Wingard (12) has already shown that the petunia is susceptible to infection by tobacco ring spot, but natural infection of this host has only recently been observed. The first case of natural infection observed on petunia was found this spring in a flower bed on a Pittsylvania County

[1] Paper No. 76 from the Department of Botany and Plant Pathology, Virginia Agricultural Experiment Station.

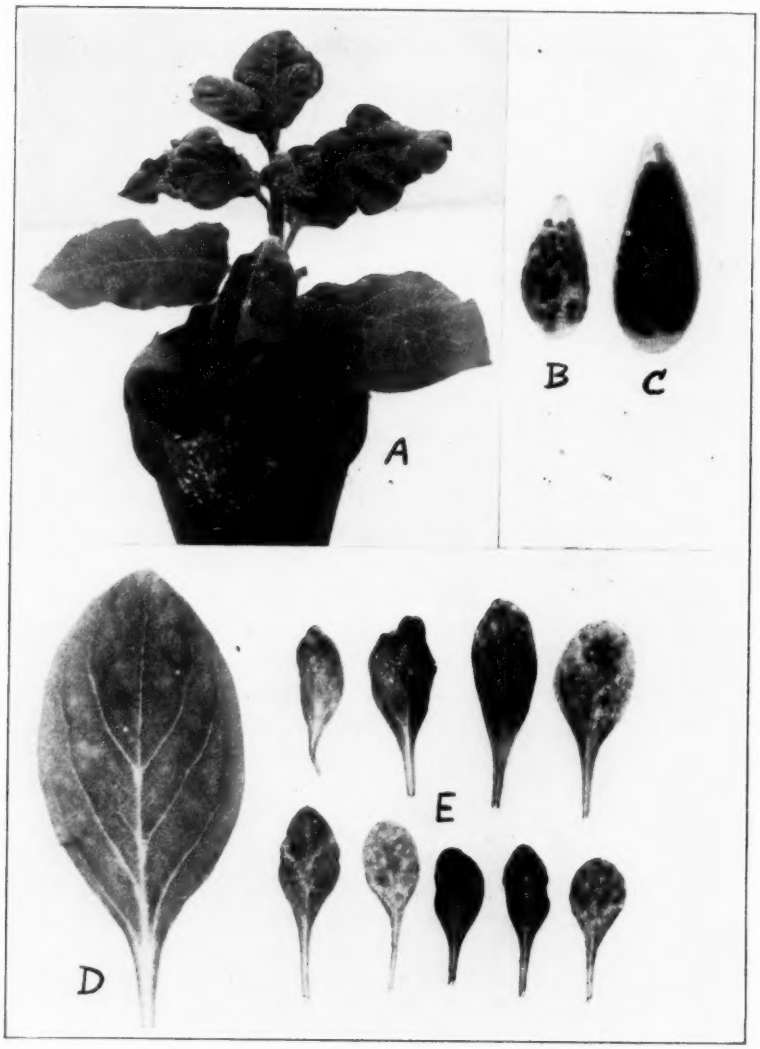

FIG. 1.—A, Turkish tobacco plant 26 days after being inoculated with expressed juice from petunia seedlings infected with ring spot transmitted through the seed. B, half of petunia seed pod from a ring-spot-infected plant. This material was killed in a solution prepared from 5 gm. mercuric chloride, 5 cc. formalin and 5 cc. glacial acetic acid in 100 cc. of 50 per cent alcohol, treated with cellulose acetate solution for about two weeks and finally cleared in oil of Gaultheria. About 2.5×. C, half of a petunia seed pod from a healthy plant, which was treated in the same manner as described above under B. About 2.5×. D, leaf from healthy petunia seedling shown in figure 2, A. About 1.5×. E, leaves from ring-spot-infected petunia seedlings. Some of these leaves were taken from the seedlings shown in figure 2, B. About 1.5×.

(Va.) lawn, and the second instance was found about a month later under similar conditions in Washington County. The symptoms of the disease on the petunia in these two instances corresponded with those described by Wingard for this plant. Of course, the fact that ring-spot infection occurred naturally on petunia in these instances does not necessarily mean that it was transmitted through the seed, but the writer feels that it is a strong bit of circumstantial evidence to that effect.

The writer's experimental results have shown very clearly that the tobacco ring-spot virus is transmitted through the seed of petunia. In August, 1929, a lot of seed was collected from a number of ring-spot-infected petunia plants growing in the Experiment Station greenhouse at Blacksburg, Virginia. These plants had been inoculated with ring-spot virus from tobacco plants. Immediately following the inoculation their growth was greatly retarded, but later the ring-spot symptoms became partially masked and the plants grew and blossomed normally. Many of the flowers, though apparently normal, remained sterile; and those that were fertile set pods that bore only a little seed as compared to a normal pod (Fig. 1, B, C).

About a month after the date of harvest, a portion of this lot of seed was sown in a greenhouse bed. The seed bed had been previously steamed and carefully prepared, and special effort was made throughout the experiment to prevent contamination from outside sources. The greenhouse was kept practically free of insects, and no ring-spot material of any kind was handled near the beds in which the petunia plants were growing. The seed germinated well and the seedlings showed no indication of damping-off or dying from any other cause.

The results of this experiment were quite surprising. Out of the 810 seedlings grown, 160, or 19.8 per cent, developed ring-spot infection. Inoculations with this material gave positive results on tobacco (Fig. 1, A), thereby demonstrating that the petunia seedlings were infected with ring spot. The infected seedlings were severely dwarfed and stunted, as shown in figure 2, B. The first few leaves were mottled in color and streaked with watery green spots and lines that more or less followed the veins (Fig. 1, E). Some of the leaves showed only a few of the watery green areas and many light brown streaks that apparently resulted from a slight necrosis of the leaf tissues. A curling of the leaves along the margin was also noticeable in some cases (Fig. 1, E).

The healthy seedlings were removed from the bed and the infected ones left and allowed to grow to maturity (Fig. 2, D). They remained dwarfed for a short time but later seemed to outgrow the disease and lose nearly all symptoms of infection (Fig. 2, C). Some of the leaves, in spite of the gen-

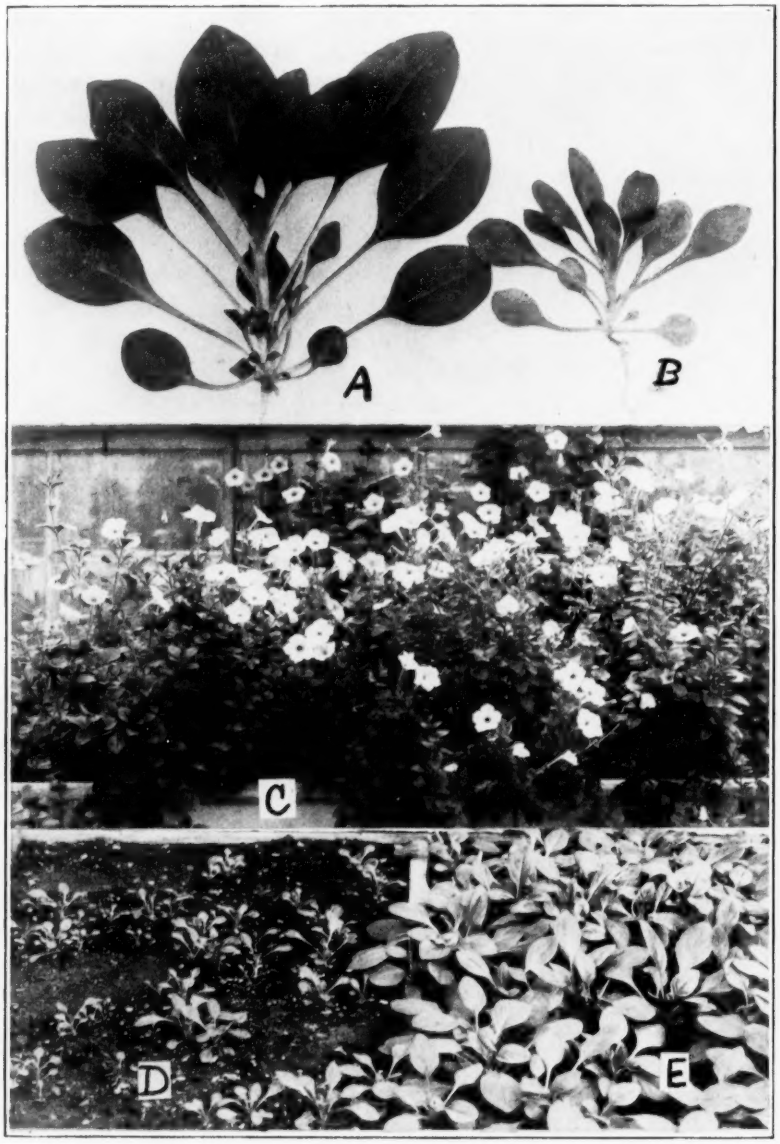

FIG. 2.—A, healthy petunia seedling. About 7/10×. B, ring-spot-infected petunia seedling the same age as the one shown in A. About 7/10×. C, ring-spot-infected petunia plants with the symptoms largely masked. This is the same bed as shown in D and E, but photographed four months later. D, ring-spot-infected petunia seedlings. The healthy plants have been removed. The seed were sown September 24, 1929, and the photographs made November 19, 1929. E, diseased and healthy petunia seedlings. The healthy plants were later removed and the diseased ones permitted to grow to maturity.

eral masking of the symptoms of infection, continued to show streaks, and many developed chlorotic and necrotic rings typical of systemic infection.

On March 24, 1930, the remainder of the lot of seed used in the experiment here reported was sown in the greenhouse. On May 5, an examination was made for ring-spot infection, and 21 out of a total of 104 seedlings exhibited symptoms typical of the disease. This is approximately the same percentage of infection as was transmitted in the previous test, and it shows that the virus did not decrease in virulency during the extended period of time in which the seed remained in storage.

VIRGINIA AGRICULTURAL EXPERIMENT STATION,
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SOLUBILITY OF BORDEAUX

GEORGE L. HOCKENYOS

Since its first systematic use by Millardet in 1883 Bordeaux mixture has been the subject of much serious study. All phases dependent on the determination of solubility have, of course, been limited to the accuracy of the analytical methods available. Up to the present the most sensitive method of determining copper has been the ferrocyanide colorimetric method, credited with detecting one part of copper in 2,500,000.[1]

A colorimetric method[2] of ten times this sensitivity has recently been published and the writer has applied it to the investigation of some doubtful assumptions now held regarding the solubility of the copper compounds in Bordeaux mixtures.

This method is essentially that originally published and consists of taking 17.5 cc. of the solution to be determined and adding successively 0.5 cc. ammonium hydroxide and 2 cc. sodium diethyldithiocarbonate. The colored solution so produced is compared with a standard similarly prepared in a Duboscq colorimeter. A suitable standard is prepared by diluting 2 cc. of a 1/5000 N solution of copper sulphate to 17.5 cc. and proceeding as above. If the sample is much darker than this it should be diluted and comparisons made between approximately similar solutions.

The writer found that where considerable amounts of calcium salts were in solution there was developed a cloudiness that interfered with the analysis. In such cases the method was modified as follows: To 15 cc. sample add 0.5 cc. concentrated hydrochloric acid to insure a clear solution; then add 1.5 cc. concentrated ammonium hydroxide and 1 cc. of a 1 per cent solution of saponin to peptize the colloids formed and finally add 2 cc. of 1 per cent sodium diethyldithiocarbonate. The standard is made up in the same way and it is essential that approximately similar concentrations of copper be used as the saponin has some color of its own that must be balanced. With the usual precautions attendant with the use of a colorimetric method excellent results can be obtained.

In all of this work the solubility determinations were at room temperature and the Bordeaux mixtures were prepared by dissolving the lime and copper sulphate separately in one half the total amount of water and then pouring the copper sulphate into the lime with vigorous stirring.

[1] Yoe, John H. Photometric Chemical Analysis, Vol. 1, J. Wiley & Sons, Inc., New York, and Chapman and Hall, Paris, 1928.

[2] Callan, Thomas, and J. A. K. Henderson. A new reagent for the colorimetric determination of minute amounts of copper. Analyst **54**: 650–653. 1929.

The first point investigated was on the solubility of metallic copper itself. Numerous workers have noted that metallic copper exerts a toxic influence when present in algal, bacterial, or fungous cultures. Nägeli,[3] being unable to demonstrate the presence of copper in solution, formulated the so-called "oligodynamic" theory.

The writer placed some copper wire, which had been cleaned by scraping with a knife blade, in distilled water. Care was used to avoid touching the wire with the hands. One sample was placed in water recently boiled to free it of gases and another in water saturated with atmospheric gases by bubbling washed air through it. The containers were then tightly corked. The percentage of copper in solution is shown in the following table:

	Boiled Water	Aerated Water
24 hours	0.00013 per cent	0.00006 per cent
3 days	0.00039 " "	0.00032 " "
9 days	0.00104 " "	0.00039 " "

The fact that more copper, ultimately dissolved in the boiled water, may be associated with the formation of an insoluble film in the presence of dissolved atmospheric gases.

Bordeaux is commonly made on a formula approximating the so-called 4–4–50 or 4–6–50 formula. These latter two formulae give 1 per cent copper sulphate to 1 per cent lime or 1 per cent copper sulphate to 1.5 per cent lime, respectively. Pickering,[4] in 1917, published a list of the compounds formed when copper sulphate and lime are mixed in various ratios varying from 1 part copper sulphate to 0.169 parts lime up to 1 part copper sulphate to 0.67 parts lime.

A series of seven mixtures were made up, five of them in the ratios indicated by Pickering and the other two in the ratios commonly used in orchard practice. In all cases 1 per cent copper sulphate was used. Thus in the case of sample F the solution was equivalent to a 4–4–50 Bordeaux. The percentage of copper in the supernatant fluid was determined after three days' settling.

In their book, "Science and Fruit Growing," Spencer and Pickering discuss these formulae as follows: A is just enough lime to precipitate all the copper present, C is a neutral Bordeaux, and, if excess lime is used, an

[3] Nägeli, Carl K. von. Ueber oligodynamische Erscheinungen in lebenden Zelle. 51 pp. 1893. Zürich.

[4] Pickering, S. V. The Chemistry of Bordeaux mixture. Jour. Chem. Soc. **91**: 1988–2001. 1907.

	Ratio copper to hydrated lime	Orchard formula	Pickering's formula	Per cent copper in solution	Color of precipitate
A	1–0.169	4–0.67–50	10 CuO 2.5 SO_3	0.04	Greenish
B	1–0.18	4–0.72–50	10 CuO 2 SO_3	0.03	"
C	1–0.203	4–0.81–50	10 CuO SO_3	0.016	Greenish-blue
D	1–0.27	4–1.08–50	10 CuO SO_3 3 CaO	0.00009	Pale blue
E	1–0.67	4–2.68–50	10 CuO 30 CaO	0.00013	" "
F	1–1.0	4–4–50		0.00014	Medium blue
G	1–2.0	4–8–50		0.00014	Dark blue

alkaline Bordeaux results with compounds C and D being formed and it is only after several days standing that compound E is formed. They further add that in the case of formula D no copper can be detected at all in solution.

In making the above analyses the writer pipetted a portion of the supernatant liquid, rather than filter it off, because it was found that filtering altered the copper content of not only the Bordeaux solution but of the standard as well. This may have been due to the absorption of the copper ions by the filter paper or to the interference of filter-paper fibrils in the formation of the colored compound. That the copper obtained in these analyses was not particles in suspension is shown by the fact that a precipitate made by formula F was repeatedly decanted with distilled water and finally washed on a filter. Upon being shaken with distilled water and allowed to stand two days, it gave 0.00013 per cent copper in solution. A further check was made by drying such a precipitate in the oven over night at 180° F. and then shaking in distilled water. After two days an analysis showed 0.00010 per cent copper in solution.

In commercial practice, stone lime or calcium oxide is sometimes used in place of the hydrated lime or calcium hydroxide. Several mixtures were made up using the chemical equivalent of stone lime in place of the hydrated form. The lime was boiled in a small amount of water and diluted before mixing with the copper sulphate. This gave a very milky lime suspension and when precipitated it gave a dense dark blue precipitate. The liquid, however, tested 0.00010 per cent copper and did not differ greatly from the hydrated lime precipitates. It was also found that if hydrated lime be boiled vigorously for a few minutes with a small amount of water before diluting and mixing with the copper sulphate solution, it tends to make a darker colored and denser precipitate than where simply shaken up in the cold.

Sugar has frequently been used as a preservative and it has been suggested that its action is due to its chemical combination with the calcium

hydroxide. Certain it is that both the precipitate and the supernatant liquid take on a deep blue color when sugar is added. A solution containing 0.5 per cent sugar was analyzed and found to contain 0.078 per cent copper actually in solution.

Numerous other applications of this method suggest themselves. Among these are the effect of atmospheric agents on Bordeaux spray on fruit-tree foliage and the effect of the excretions of the leaves themselves. Some studies of these factors were recently made by DeLong[5] but this method was not used.

DEPARTMENT OF HORTICULTURE,
UNIVERSITY OF ILLINOIS,
URBANA, ILL.

[5] DeLong, D. M., W. J. Reid, Jr., and M. M. Darley. The plant as a factor in the action of Bordeaux mixture as an insecticide. Journal Economic Entomology 23: 383–390. 1930.

THE AVOCADO DISEASE CALLED SUN BLOTCH[1]

WM. T. HORNE AND E. R. PARKER

The writers are not aware that the avocado disease called sun blotch occurs in any country except California.[2] We do not have accurate information as to when it was first observed in this State, though it is now frequently met with and may be considered of general distribution here.

The first published mention of the disease which has come to our attention is the article by Dr. Coit[3] where a brief, but effective, description of the disease is given with five illustrations. The nature of the disease is indicated and an interesting hypothesis set forth as to its cause, and, finally, the most important procedures as to control are pointed out.

As indicated by Dr. Coit, symptoms of sun blotch involve the fruit, young stems, branches in general, and trunk. On the fruit, long and narrow, shallow, longitudinal grooves or depressed streaks appear near the stem end (Fig. 1). The surface of the streak tends to be smooth. If the fruit is short and broad the streak tends to be a smooth, flattened or somewhat depressed area radiating from the vicinity of the stem end. The color of the fruit streak in green fruits is whitish or yellowish, whereas with purple varieties affected areas develop striking red or purple red colors.

On the young stems shallow, light-color or, in some varieties, buff-color longitudinal grooves or streaks may appear (Fig. 2). Some bright red streaking may show also in the stem lesions. Where symptoms are well developed they are striking, but frequently the streaks fade out to merely obscure mottling; also excessively vigorous normal shoots have certain longitudinal grooves and ridges. As heavily affected stems become somewhat older they become very uneven and rough with prominent lenticels. The shoots become decumbent, somewhat twisted and abnormal in appearance, having a dull color and unthrifty aspect. In addition to the symptoms described by Dr. Coit we have observed on the variety Caliente a white variegation of unequal distribution on the leaves. This appears to be a symptom of sun blotch. Bark of the trunk of sun-blotch trees has been observed to be unusually rough, but we are not sure that this is an invariable indication of the disease.

[1] Paper No. 228, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

[2] Dr. H. S. Fawcett, in a personal letter written from Rome on June 11, 1930, states that he saw sun blotch in a tree or two in Palestine, trees which he understood came from California.

[3] Coit, J. E., Sun blotch of the avocado, a serious physiological disease. Yearb. Calif. Avocado Assoc. 1928: 27–32. 1928.

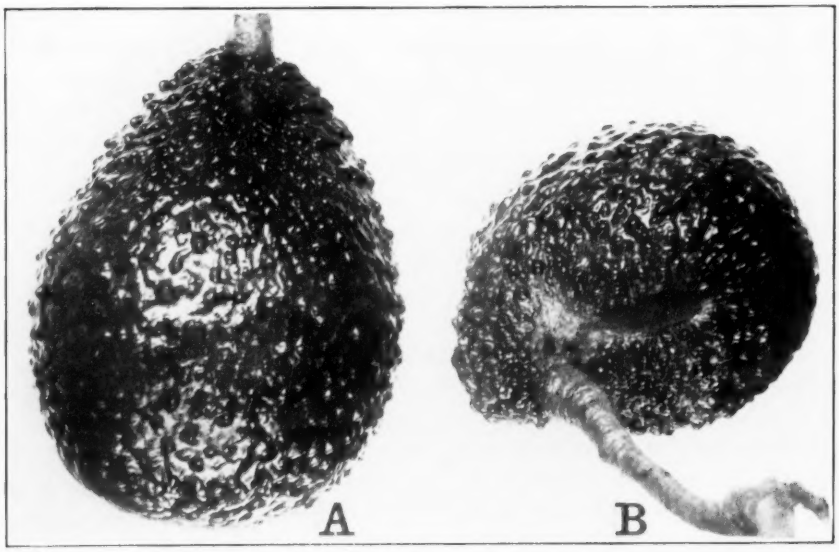

FIG. 1. Sun blotch in avocado fruits of the Lyon variety. A, a pronounced longitudinal crease near the stem (see arrow) which is sufficient to disqualify the fruit for first grade. B, broad, deep depressions near the stem, causing serious distortion. In one of the sun-blotch depressions is an area of secondary necrosis.

A striking feature appears to be the variation in the degree to which symptoms are shown. Some trees have undoubted symptoms of the disease yet appear normal, except on close examination, and it is probable in some cases that affected trees cannot be recognized even by experienced persons. In other cases affected trees become very abnormal and worthless. Severe pruning appears to accentuate symptoms on the robust shoots. It seems probable that the appearance of symptoms of the disease and their severity are related to the vigor of growth of the affected part.

The disease has been observed on numerous varieties and none are known to be immune. Importance of the disease is as yet uncertain. If it should develop extensively in its more severe form, as it appears on unthrifty nursery trees, it would certainly be disastrous. On some large trees the only injury discoverable is an occasional slightly marked fruit which becomes a cull, general vigor and production being fairly normal.

In the affected parts of the plant there appears to be a deficiency in the development of certain tissues but the pathological histology has not yet been worked out.

The suggestion made by Dr. Coit as to the cause of sun blotch, *i.e.*, that it originates where tissue is injured by sunburn, is interesting. Whatever the origin, it seems clear that sun blotch, once originated, may be continued

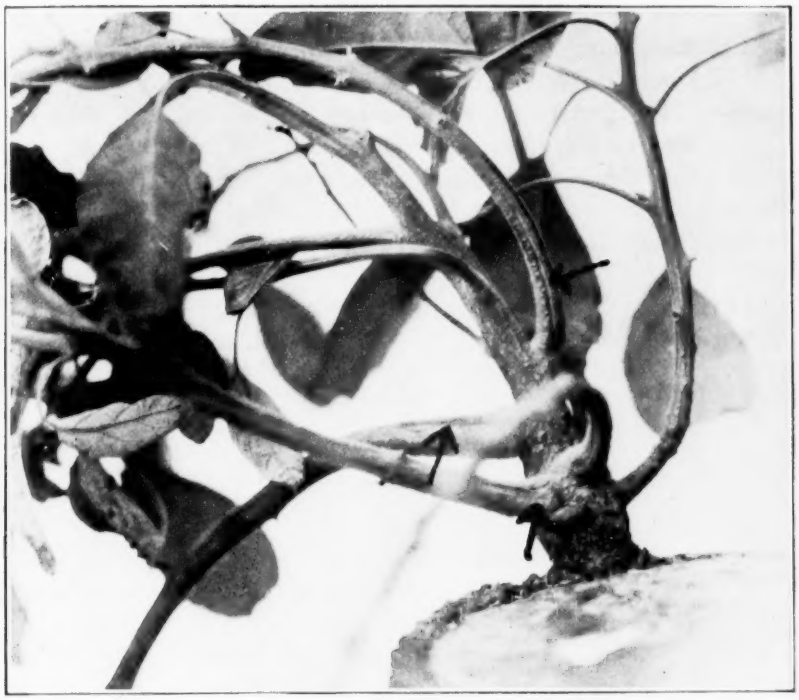

FIG. 2. Sun-blotch scion on healthy stock showing beginning of the decumbent habit, grooves on the twigs (see arrows), and rough abnormal bark.

by budding or grafting and is then not dependent on sunburn for its development.

It is well recognized by practical men that a sun-blotch scion set in a normal tree grows with the sun-blotch character. Scions set by the second writer at the University of California Citrus Experiment Station, Riverside, Calif., have shown this behavior. In the case of a tree with only slight symptoms of sun blotch, we are not sure that all twigs would, if used as scions, transmit the symptoms to shoots originating from them.

Dr. Coit gave the writers certain evidence indicating that where a sun-blotch-diseased scion grew on a healthy stock shoots subsequently arising from the stock (Fig. 2) also might show the disease. In at least three cases this has clearly occurred at the Citrus Experiment Station. The disturbed condition, therefore, progresses from diseased scion to healthy stock. Growers state that healthy scions set in sun-blotched trees grow out with the symptoms of sun blotch. Owing to the difficulty involved in making sure that a given scion is free from sun blotch, this point is receiving further study.

Inoculations on seedlings have been made in several ways: by needle pricks, first moistening the needle with juice of affected tissue; by hypodermic needle, introducing juice from diseased tissue; by hypodermic needle, using juice from diseased tissue which had been passed through a Berkefeld filter; and by introducing pieces of living diseased tissue. Results from these inoculations are negative to date. Through the kindness of Professor E. B. Babcock a set of plants was treated with X-rays, but it is not yet apparent that any permanent change in the tissues has been induced.

From the above evidence it would appear that the disease of avocados called sun blotch belongs to the group of plant diseases called infectious chloroses.

DOWNY MILDEW OF SORGHUM AND MAIZE IN EGYPT[1]

L. E. Melchers[2]

The downy mildew of sorghum and maize was found by the writer in June, 1928, on the agricultural experimental farm belonging to the Ministry of Agriculture, Giza, Egypt. It had not previously been reported in Northern Africa. The disease was first noticed on plants of sorghum (durra) as they approached the heading stage in a "date-of-planting" experiment which had been made for the purpose of studying the effect of soil temperature on kernel-smut (*Sphacelotheca sorghi* (Lk.) Cl.) infection. All plantings became attacked by the downy mildew. Later, the disease appeared on some maize which grew adjoining the durra plots.

As this was the first time downy mildew had been observed in Egypt, the matter was of considerable importance because of the place which durra, and especially maize, hold in Egyptian agriculture. The various infected plantings were immediately ordered removed and burned, and close watch was kept on all other plantings in the vicinity. Downy mildew appeared later in another section of the farm on some sorghum varieties, the seed of which the writer had brought from Kansas. All plants were removed as soon as they were found to be infected. Several scouting parties were sent to other Provinces where sorghum (called millet in Egypt) is grown to search for the presence of downy mildew. Fortunately, it was not found at that time on sorghum or maize in any other Province except at Giza.

The source of the infection by this fungus is not definitely known. Circumstantial evidence indicates that it may have entered Egypt on packing materials from India. The Section of Horticulture at Giza imports plants from India and these may have been the source of the inoculum. The writer examined the grasses in the vicinity of the Giza farm, believing that infection very probably originated from such a source, but no evidence of its presence on grasses was discovered. It is possible that if additional time had been given to a more comprehensive search of the wild grasses of the tribe Andropogoneae of the Gramineae, its presence would have been found; at least, this is the logical place to have expected its occurrence. The entire matter of the origin of the fungus in Egypt remains problematical.

During the latter part of June, and July, August, and September, when maize and sorghum are growing, the climate of the delta is generally warm

[1] Contribution No. 309 from the Department of Botany and Plant Pathology, Kansas Agricultural Experiment Station.

[2] Chief Mycologist, Egyptian Ministry of Agriculture, Cairo, Egypt, 1927 to 1929.

and humid; therefore, conditions are favorable for the development and spread of downy mildew.

Material was examined microscopically by Dr. R. M. Nattrass, one of the assistants of the Mycology Division, who identified the organism as *Sclerospora graminicola* var. *andropogonis sorghi*. Examinations by Dr. Nattrass and myself of the downy mildew on maize led us to believe that it was the same species. No striking differences in appearance or measurements could be found between the conidial stages on durra and on maize. Apparently it is the same species as that present on sorghum in India. If it is the same species on sorghum and maize in Egypt, the matter is of considerable scientific interest, as all literature describes the species attacking maize as an entirely different species and there seems to be no record of the same species attacking both maize and sorghum.

Upon returning to the United States, the writer sent specimens on sorghum to W. H. Weston, Jr., who kindly examined the material and compared it with specimens from India, Java, and South Africa. As a result of his examinations he has informed the writer that he would identify the Egyptian form on sorghum as *Sclerospora graminicola* var. *andropogonis sorghi* of Kulkarni. According to Dr. Weston the conidial stage present in this Egyptian material is not sufficiently mature to show certain features important in diagnosis, but the resting spores (oogonia with oospores) are fairly abundant and well developed. These bodies, in diverse collections of this Sclerospora from sorghum, show some variation. In essential characteristics of size, structure, color, etc., this Egyptian material most closely resembles the specimens from Kirkee, India, rather than those from Poona, India, where Kulkarni originally established the variety.

A more complete ecological study of this problem in Egypt would have been extremely interesting, together with cross-inoculation studies on durra and maize. This was not attempted because of attending dangers. Every effort was made to eradicate this disease as soon as it was discovered, as there was no way of knowing how rapidly it might spread. In the writer's opinion, there is circumstantial evidence to indicate that downy mildew may have been present on sorghum plantings at Giza in 1926 but was not recognized as such by the mycologist in charge. Although every precaution was taken to prevent this disease from becoming established in Egypt, there is some doubt whether downy mildew of sorghum or maize would find the proper environmental conditions to produce epiphytoties in that country.

KANSAS AGRICULTURAL EXPERIMENT STATION,
MANHATTAN, KANSAS.

PHYTOPATHOLOGICAL NOTES

Another host for Ustilago striaeformis (Westd.) Niessl.—In May, 1929, the writer found a plant of bottle-brush grass, *Hystrix hystrix* Millsp., heavily infected with a leaf smut. The plant was growing in a yard at Manhattan, Kansas, and undoubtedly was from seed scattered by a plant that had been grown, the preceding season, as an ornamental in the garden. Many other seedlings of the same species were growing within a few feet of the smutted plant, but none of them showed any infection.

Leaf smuts of grasses are seldom seen in the vicinity of Manhattan, so the infected plant was carefully transplanted to a pot in the greenhouse where it could be watched. Here it grew to normal maturity and produced a spike typical of *Hystrix hystrix*. When found the plant had only four or five leaves, all of which, except the first two, showed the long grayish black lesions characteristic of this leaf smut on other grasses. All subsequent leaves showed many such lesions. As is typical of most leaf smuts on grasses, the sori at first were covered by the epidermis, but as the leaves matured, the epidermis ruptured and exposed the black spore masses.

At maturity the smutted leaves were collected and a specimen sent to the Office of Mycology and Disease Survey, United States Department of Agriculture, where the fungus was identified by R. W. Davidson as *Ustilago striaeformis* (Westd.) Niessl. Another specimen was sent to Dr. A. G. Johnson of the Office of Cereal Crops and Diseases who, together with Mr. W. W. Diehl, also identified the fungus as *U. striaeformis*. Reports from both sources stated that no previous record of the occurrence of *U. striaeformis* on *Hystrix hystrix* could be found. Therefore, it seemed desirable to record *H. hystrix* as another host for this smut. The other plants of this grass, growing within a short distance of the smutted plant, were observed at frequent intervals throughout the summer of 1929, but none of them developed the smut. Seedlings from those plants emerged in the spring of 1930, but, out of more than 100, none have shown signs of leaf smut.—C. O. Johnston, Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, cooperating with the Kansas Agricultural Experiment Station, Manhattan, Kansas.

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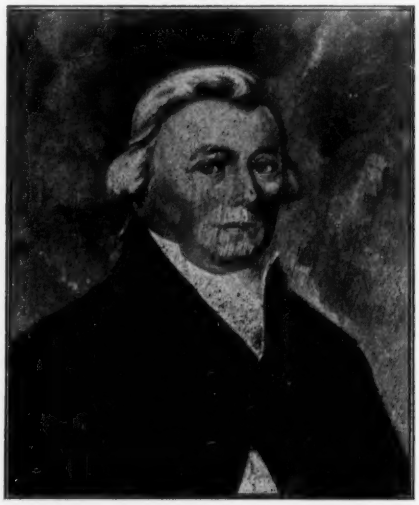

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